Social Communication In Domestic Horses: The Production And Perception Of Facial Expressions.

Dissertation submitted to the University of Sussex for the degree of Doctor of Philosophy.

Jennifer Wathan

June 2015

I herby declare that this thesis has not been and will not be, submitted in whole or in part to another University for the award of any other degree.

Signature……

Jennifer Wathan

Acknowledgements

First, I would like to thank my supervisor, Karen McComb, for offering me the opportunity to study for this PhD, and for her faith, help, and guidance throughout every stage of this process. I would also like to thank Katie Slocombe, without whom I may well never have begun the formal study of animal behaviour.

Countless people assisted me with collecting the data presented in this thesis, including those who allowed me access to their horses - some of the most notable being the staff and volunteers at Albourne Equestrian Centre, Bowler's Riding School, CHET Centre, Ditchling Common Stud, Glebe Field Equestrian Centre, Hope in the Valley Riding for the Disabled, Plumpton College, and The Diamond Centre. Charlotte Lillis, Becky Spooner, and Chris Wathan also helped considerably in the data collection. Anne Burrows and Bridget Waller were fundamental to the development of EquiFACS. I thank Bridget for her advice in adapting FACS for a non-human animal, and Annie for sharing her anatomical expertise. The school administration team and technicians were always willing to offer their assistance during my studies. My office mates, Thibault Chabert, Kate Grounds, Leanne Proops, Jordan Raine, Graeme Shannon, and Amy Smith offered me their friendship, and Kate, Leanne, and Amy have also provided valuable support in innumerable other ways.

Thank you to all my friends and family for their love and support along the way. Emma Green deserves a special acknowledgement for her help in proof reading. Andy Bamford has contributed to greatly to this thesis through providing endless discussions and advice, practical help whenever I was short of assistance, and trips to Madagascar for cherished adventures, if not quite rest and recuperation. A final mention must be given to Barty - for his critical judgement on my experimental protocols, and for constantly reminding me of how much more there still is to learn about how horses think.

This research was funded by a studentship from the Biotechnology and Biological Sciences Research Council and the School of Psychology, University of Sussex. A University of Sussex Alumni Travel Grant supported the collaboration with Anne Burrows, which contributed to Article I.

Author Contributions

The thesis conforms to an 'article format' in which the empirical chapters consist of discrete articles written in a style that is appropriate for publication in peer-reviewed journals in the field. The first and final chapters present an introduction to and discussion of the field and the research undertaken. I am the principle author on the manuscripts that form this thesis, and have been responsible for all aspects of the design, implementation, analysis, and dissemination of this research. Karen McComb is listed as senior author on all of the articles to acknowledge the contribution of her expertise and advice to all stages of my work. Other specific author contributions are described below.

Article I: Wathan, J., Burrows, A.M., Waller, B.M., & McComb, K. EquiFACS: The Equine Facial Action Coding System. *PLOS ONE,* in press.

AMB contributed to the dissection and recording of the facial musculature of the horse, and identifying the muscular basis of horse facial movements. AMB also provided vital reagents and tools, and provided feedback on the manuscript draft. BMW provided feedback on the study design, assisted in identifying the muscular basis of horse facial movements and their relations to previous FACS, and the manuscript draft.

Article IV: Wathan, J., Proops, L., Grounds, K., & McComb, K. Functional use of Facial Expressions in a non-primate: horses discriminate between differing emotions. *In the Style of Proceedings of the Royal Society B: Biological Sciences.*

LP and KG contributed to the design of the experimental protocols, assisted with data collection and analysis, in particular the video coding and heart rate analysis, and provided feedback on the manuscript draft.

University of Sussex

Jennifer Wathan Doctor of Philosophy

Social Communication In Domestic Horses: The Production And Perception Of Facial Expressions.

Summary

Living in complex societies is thought to promote the development of sophisticated social, cognitive, and communicative skills. Investigating the extent of these skills across taxa is critical to understanding the evolution of the advanced abilities found in some species, including humans. Facial expressions are rich sources of social information for humans and some primates; however whether this is true for other animals is largely unknown. Horses are an ideal study species for these questions: they form valuable social relationships and display some advanced socio-cognitive skills, but are phylogenetically distant from primates and so might be expected to communicate quite differently. Here I present a method for quantifying and coding horse facial movements (EquiFACS), which reveals that horses have an extensive capacity for producing facial expressions. I then utilise EquiFACS to demonstrate that horses produce facial actions that mirror the emotional content of auditory stimuli, providing evidence for a perception-action representation of emotional information. Through my experiments on the perception of facial expressions in horses I show that these expressions display meaningful information to conspecifics, which influences their behaviour in functionally relevant ways. I also shed light on the physiological processes involved in the perception of emotional conspecific facial expressions, showing that viewing negatively valenced conspecific emotional expressions raises resting heart rate. This is indicative of emotional contagion, which may be a mechanism through which information is obtained and social interactions are regulated. Collectively, my research demonstrates the ability to produce and use complex facial expressions as a source of social information is not limited to primates, but is present in at least two phylogenetically distant groups. This suggests these skills may either be an evolutionarily conserved trait or have evolved under common selective pressures. As well as their scientific significance, these findings have implications for horse management and welfare.

Table of Contents

List of Tables and Figures

Introduction

Many characteristics that were once considered uniquely human (*Homo sapiens*) are now being demonstrated in other animals. This includes neurophysiological structure, (e.g. cortical lateralisation, Farmer et al., 2010), sophisticated behaviours, (e.g. toolmaking, Goodall, 1986), and cognitive concepts, (e.g. understanding that others have differing knowledge and desires to one's own, Premack and Woodruff, 1978). This is perhaps unsurprising given that evolution involves a gradual series of small modifications to existing abilities and in line with this, it is now well recognised that evolution is not a case of humans having reached the top of an evolutionary 'tree' – rather, that organisms have diversified in interesting ways to adapt to their own environments (Hecht et al., 2012). Examining where these adaptations have converged, as well as their diversity, can provide insights into how particular abilities may offer an advantage and the selective pressures that may have underpinned their development. Ultimately, the study of a wide range of animals can help us to understand the evolution of the advanced abilities found in some species, including humans.

Social Cognition

What is social cognition?

Brain size is often used as a proxy for cognitive capacity, as it offers an index that is directly measurable and comparable across species (Byrne, 1995). However there are physical and physiological constraints on absolute brain size; for example, as body size increases metabolic efficiency also increases, which provides more energy for foetal brain growth and allows the development of large brains (Armstrong, 1982, Martin, 1981). Indeed, physiological and life history traits including large body size, high metabolic rate, and prolonged development are closely associated with large brains (Armstrong, 1983, Harvey and Clutton-Brock, 1985, Perez-Barberria and Gordon, 2005). For this reason the ratio of brain to body size is an interesting value, and a number of studies have demonstrated that some animals have particularly large brains for their body size (Byrne, 1995). The brain is one of the most energy demanding organs, with brain tissue being metabolically costly to maintain (Aiello and Wheeler, 1995, Kaufman, 2003). As such, presumably there must be some advantages that outweigh the costs of having a large brain.

Traditionally, the study of animal behaviour looked to the physical environment to explain differences in brain size and cognitive capacity. Among primates (*Primates spp.*), those with larger home ranges, which may have required more sophisticated navigation, had larger brains (relative to body size). Similarly, frugivorous primates tend to have larger brains than folivores, purportedly because fruit is a more unpredictable food source than leaves and thus may require more advanced cognitive skills (e.g. identifying the location of the fruiting trees in the correct season, Clutton-Brock and Harvey, 1980). However, folivores need larger bodies than frugivores for adequate digestion, so this may be a confounding variable in the brain-body ratio (Byrne, 1995). Additionally, some animals with much smaller brains than primates also face difficult foraging decisions, such as squirrels (*Sciuridae spp.*) that have to remember the location of cached food stores (Mace et al., 1981). Consequently, ecological complexity alone does not seem to adequately explain the evolution of large brains and sophisticated cognition.

For social species, other individuals constitute a large component of the environment. In a wide range of animals including primates, ungulates (*Ungulata spp.*), carnivores (*Carnivoria spp.*), cetaceans (*Cetacea spp.*), and some insects (*Insecta spp.*), relative brain size correlates with multiple indices of social complexity (Connor et al., 1998, Dunbar and Bever, 1998, Dunbar, 2003, Dunbar and Shultz, 2007, Ehmer et al., 2001, Julian and Gronenberg, 2001, Marino, 2002, Perez-Barberria and Gordon, 2005, Pérez-Barbería et al., 2007, Shultz and Dunbar, 2006, Shultz and Dunbar, 2010). In particular, increased encephalisation over time is associated not only with sociality but specifically with living in stable social groups (Shultz and Dunbar, 2010). Consequently, there is growing support for the suggestion that a key selection pressure promoting the evolution of large brains and advanced cognition is explicitly social (Byrne and Whiten, 1988, Humphrey, 1976, Jolly, 1966). Social cognition refers to the skills that are used to navigate the social environment including how animals process, store, and utilise information about other animals. The study of social cognition across species will contribute to understanding whether the need for these skills had a role in the evolution of large brains.

Living in a complex society

Animals have different levels of interaction with members of their own (and other) species. Some species live fairly solitary lives but many are highly social - feeding, travelling, and resting with conspecifics. In these social species, individuals show a strong need to remain in groups and maintain group cohesion, even though they are competing for limited resources and may suffer costs as a consequence (Dunn et al., 1995, Pusey and Packer, 2003). However group living can afford several advantages, including decreased predation, increased reproductive opportunities, enhanced foraging efficiency, and co-operative rearing of offspring (Brown, 1988, Clutton-Brock et al., 2001, Lima and Bednekoff, 1999, White and Galef, 2000). These benefits can be conferred through relatively simple mechanisms; for example, the presence of a foraging animal may increase the interest of others in that area, acting as a form of local stimulus enhancement (Pöysä, 1992, Valone and Templeton, 2002, Valone, 1989). However at a more complex level, sociality also provides animals with the opportunity for social learning, which avoids costly trial and error and allows an accumulation of knowledge, potentially over generations (Whitehead and Rendell, 2014, Whiten and van Schaik, 2007).

In addition to the potential benefits that increased group size can offer, the formation of strong and stable social bonds has direct fitness benefits in a number of species (Cameron et al., 2009, Nunez et al., 2014, Silk et al., 2010, Silk et al., 2009). However, forming and maintaining these valuable bonds potentially presents a cognitive challenge, particularly in the face of competition for limited resources. In societies that involve several long-term relationships this must at least require a high working memory load (Byrne and Whiten, 1988). Moreover, animals have continuously and rapidly changing needs and motivation, so behavioural strategies for dealing with other individuals must be flexible and presumably a sensitivity to their internal states would be advantageous (Humphrey, 1976). Subsequently, the ability to gather, retain, and use detailed social knowledge about multiple individuals and the development of elaborate and varied communicative repertoires are thought to depend on the social complexity of the species and the quality of relationships between individuals (Byrne and Bates, 2007, Byrne, 1995, Aureli et al., 2008, Cheney and Seyfarth, 2008, McComb and Semple, 2005, Premack and Woodruff, 1978).

Some societies are characterised by having a large community that is comprised of several smaller groups, which regularly come together and part in a fluid and changing manner (fission fusion dynamics). Maintaining relationships under these circumstances is arguably more challenging than in groups where the composition of the social environment is more stable. In fission fusion societies individuals have to maintain relationships with numerous others based on irregular and infrequent interactions. Additionally, it may be beneficial for individuals to monitor third party relationships that might influence them or their group as a whole, yet they may not be able to observe all of the interactions that occur (Aureli et al., 2008). Having the ability to hold in mind earlier events, form mental representations of individuals not currently present, and predict the outcome of changing scenarios involving numerous unpredictable social partners represents a serious cognitive challenge (Barton, 2006).

Clearly, group living creates challenges for successfully navigating the social environment, and dealing with social problems may be one of the biggest cognitive hurdles faced by animals (Aureli et al., 2008, Humphrey, 1976, Jolly, 1966). Living in an extensive social network correlates with, and is likely to select for, cognitive sophistication and advanced communication (Byrne and Bates, 2007, Dunbar, 1993). Many prominent primatologists argue that primate social groups are unique in their complexity, and thus have led to advanced and distinct cognitive and communicative capabilities in primates (e.g. Dunbar, 2003). However, other animals also display fission fusion dynamics and sophisticated social knowledge, including corvids (*Corvidae spp.*), spotted hyenas (*Crocuta crocuta*), African elephants (*Loxodonta africana*), cetaceans, and horses (*Equus caballus*) (Aureli et al., 2008, Cheney and Seyfarth, 2008). If the ability to recognise and monitor other individuals' social relationships confers a selective advantage, then we should expect to find these mechanisms in any animals that live in large stable social groups and particularly in those where valuable social relationships need to be maintained in a complex and fluid social environment.

Studying social cognition and communication

Studying how animals interact and communicate with conspecifics offers a method for gaining insights into how they understand and perceive their social world (Cheney and Seyfarth, 2008, Seyfarth et al., 2010). In particular, experimental paradigms that allow

the systematic presentation of signals and observation of the recipient's behaviour can offer insights not only into the perception of these signals by receivers, but also the selective value and evolution of the cognitive mechanisms underpinning these communicative interactions. Designing such experiments is particularly challenging in the field of social cognition, where the stimulus to be presented is not a traditional puzzle box that can be manufactured in a workshop, but is a living, breathing organism with its own intentions and desires, which may not align with rigorous and precise experimental protocols.

One way to overcome these challenges is the presentation of animal models. Playback experiments of previously recorded vocalisations have been particularly successful. These can be conducted in a naturalistic manner that allows realistic simulation of a variety of situations, including the presence of another animal and interactions between individuals (McComb et al., 2001, Seyfarth et al., 2010). Research in the vocal modality has been facilitated by well-developed knowledge about the anatomy underpinning the production of calls, and advances in digital techniques for signal analysis and manipulation (Taylor and Reby, 2010). However, presentation of animal models in the visual modality has been more challenging.

Live animal models can be used, although there is a significant challenge in achieving an experimental set up where the signal producer is giving the signal while the signal receiver is available and attending to receive it. It is also typically difficult to control the signals given or to perform any systematic manipulation. One exception is the use of human models, which have been used to great effect in investigating questions about the effects of domestication on cognition (Hare et al., 2002, Hare et al., 2005, Hare and Tomasello, 2005). However, understanding the communicative intent or internal state of another is considered a complex cognitive mechanism (Premack and Woodruff, 1978), which is surely more difficult interspecifically. If intraspecific communication systems are not well understood then it is difficult to make inferences about the true abilities of animals or explain apparently inconsistent results when they occur with human models giving signals (e.g. Proops et al., 2013, Proops and McComb, 2010, Proops et al., 2010, Rosati and Hare, 2009, Tempelmann et al., 2011).

Another option is virtual models, such as stuffed or robotic animal models, which have been very successful in certain situations but also have their limitations, particularly with large mammals (Lack, 1943, Partan et al., 2009). However, recent advances in digital photography have created new opportunities for realistic depictions of conspecific visual signals that can also be precisely manipulated. This has been particularly well developed in computer-based tasks with captive primates, and has enhanced our understanding in a number of areas, such as primate face processing (Parr, 2011). The ability to present visual signals in such a systematic way has the potential to greatly advance our knowledge of the perception of stimuli by animals. To progress we now need to develop innovative naturalistic studies that allow us to take these experiments out of the lab and ask questions not only about the perception of visual conspecific signals, but how the information gained from them may be functionally relevant.

Section summary

There is growing support for the suggestion that a need to maintain valuable social relationships in complex and fluid social environments has promoted the evolution of advanced cognitive and communicative abilities. Understanding the extent and range of these abilities in a wide range of taxa with varying ecological and social environments will contribute to our understanding of the selective pressures that promoted advanced social cognition and the advantages it may confer. Horses present an excellent study species for exploring the evolution of social intelligence. As grassland herbivores they have a relatively simple foraging environment, yet they have rich social lives and form valuable and long-term social relationships with close associates within a larger herd.

In this thesis I contribute to our knowledge of social cognition and the evolution of advanced socio-cognitive skills by investigating the communicative repertoire of horses in a modality that has previously been neglected in non-human animals: facial expressions. In the empirical chapters I document the capacity for horses to produce facial expressions in a way that allows direct comparisons with other species and then use this method to record the production of facial expressions in response to conspecific vocalisations. I then go on to use visual stimuli in a series of experiments, first validating the use of photographic models as a methodology for horses, then

investigating the perception of conspecific facial expressions in two key areas of social cognition: attention and emotion. In the following introductory sections I outline the relevant research within these fields and highlight any current controversies and unanswered questions, in addition to providing some background information about my study species, the domestic horse.

Facial Expressions

Faces are a rich source of public information, which is available to others whether purposeful or not on the part of the receiver (Danchin et al., 2004). Humans can infer age, sex, and even levels of health from a face, and we assess this information remarkably quickly. We also promptly evaluate the familiarity of the face, and if we have previously encountered the individual then we can access our previous knowledge and experience of them (Bruce and Young, 2013, Hole and Bourne, 2010, Whitehead et al., 2012). Mammals have a network of facial muscles with extensive connections to the skin and superficial fascia, which enables these muscles to create observable changes in the shape of the face (Liem and Walker, 2001). These muscles have a common innervation (the seventh cranial nerve, cn7) and although they may be involved in functions such as respiration and mastication their primary role in mammals is to create facial movements (Darwin, 1999, Schmidt and Cohn, 2001). Some mammals have a number of discrete muscles that allow specific areas of the face to be moved in isolation or in varied combinations, creating an extensive and potentially complex facial repertoire. Other mammals show fewer individual, discrete facial muscles, and present with flatter, sheets of muscle that allow less range and specificity of facial movement. Furthermore, within muscles the sophistication of the movement will vary; from muscles that have a crude on or off function to muscles that enable a subtle and sophisticated range of movements (Andrew, 1963a, Diogo & Wood, 2012, Dobson, 2009a, Huber, 1931).

The production and perception of varied facial expressions according to social context are valuable skills. Facial displays can contain information pertaining to another's internal state, potentially providing clues to their likely intentions and motivation and thus making their subsequent behaviour more predictable (Andrew, 1963a, Parr et al., 2005). Consequently, facial expressions have the potential to facilitate bonding and help

maintain relationships and group cohesion – critical functions for social animals (Dobson, 2009a, Dobson, 2009b, Flack and de Waal, 2007, Parr, 2003, Waller and Dunbar, 2005). In humans, loss of facial expression after facial paralysis or producing the wrong facial expression in an interaction (e.g. smiling in an unhappy situation) can lead to negative social consequences, demonstrating the potential adaptive value of facial expressions (Schmidt and Cohn, 2001). There is also some evidence to suggest that the production of facial expressions can mediate social interactions in non-human animals (Flack and de Waal, 2007, Preuschoft and van Hooff, 1997, Waller and Dunbar, 2005). However, despite this likelihood that facial expressions may have a critical role in social species, studies of facial expressions in animal communication research have been largely overlooked (Slocombe et al., 2011, Waller and Micheletta, 2013) and there was previously no direct evidence of non-human animals using facial expressions in a functionally relevant way.

The evolution of facial expression

More than a century ago Darwin published his work on The Expression of the Emotions in Man and Animals (Darwin, 1999). In this, Darwin described the facial displays of humans and non-human primates, dogs (*Canis familiaris*), and cats (*Felis catus*) (with some discussion of other animals) and offered some key insights: facial expressions convey information about the internal state of the producer; and we cannot understand human facial expression without also understanding the expressions of other animals. Darwin noted that there was some continuity between the facial displays of different species; however since then scientific work on facial expressions has predominantly focussed on humans and other primates. Even within non-human primates facial expressions are neglected compared to vocal or gestural communication, and are significantly less likely to be studied through experimental research (Slocombe et al., 2011), potentially due to some of the methodological limitations in presenting visual animal models discussed previously.

Additionally, it is difficult to articulate and reliably describe facial expressions as Darwin noted, “a difference may be clearly perceived, and yet it may be impossible, or at least I have found it so, to state in what the difference consists” (p13). In part, this is because facial expressions are not given as synchronous, discrete signals (although

often described as such) but are composed of multiple individual facial movements that are likely to have differing but overlapping onsets. Additionally, facial expressions are perceived categorically, where numerous distinct visual images are assigned to one category, e.g. an emotion (Bruce and Young, 2013, Hole and Bourne, 2010). This leaves the recording and analysis of facial expressions open to a large degree of observer bias, and the perceived context of an expression can influence the perception of the actual facial movements (Waller et al., 2007). Consequently, to advance our knowledge of facial expressions and their potential communicative value, frameworks that quantify behaviour and allow reliable, objective measurements are needed.

Darwin was greatly influenced by the work of Guillaume Duchenne, who studied the physiology of the facial muscles and how their contractions caused observable changes in the skin (Parent, 2005). Knowledge of the physical properties of the facial muscles can provide a basis for understanding the potential range of movements of the face, and expressive signals can then be studied and quantified in terms of their physiological underpinnings (Bolwig, 1964, Ekman et al., 2002, Ekman et al., 1990, Parr et al., 2007, Seyfarth and Cheney, 2003, Vick et al., 2007). This has been notable with the development of the human Facial Action Coding System (FACS), which is a method for objectively recording facial movements based on the contractions of the underlying facial muscles (Ekman et al., 2002). This anatomically based approach has significantly advanced our ability to explore the production, perception, and function of facial expressions in humans (e.g. Waller et al., 2008a) and is now successfully being applied to other animals, allowing us to make direct cross-species comparisons (Waller et al., 2014). Consequently, systematic examination of the structure of the facial musculature in a range of species has the potential to provide insights into the importance of facial expressions in different taxa, and is a useful framework for understanding the functional significance and evolutionary origins of this form of communication.

In mammals the facial musculature has specialised to varying degrees. Traditionally this was thought to follow phylogenetic relationships, based on seminal work comparing the facial anatomy of primates (Andrew, 1963a, Gregory, 1929, Huber, 1931). This proposed two key points: (1) that the facial musculature of the primates was more complex and more able to produce facial expressions than the facial musculature of any other taxa and (2) that within the primates, there was a “linear, ascending scale” of

complexity towards humans (Huber, 1931, p18). In line with this, there still remains a prominent opinion that primates use their facial expressions in a unique way compared to non-primate orders, with other animals only having a limited capacity for facial expression (Boissy et al., 2011, Burrows, 2008, Dobson, 2012, Tate et al., 2006). However, because research investigating the production of facial expressions has been biased towards primates, the true ability of other species remains largely unknown (Waller and Micheletta, 2013, Burrows, 2008). Moreover, recent work repeating these comparisons of primates using more modern techniques has challenged the traditional thinking.

Extensive similarity in the form and movement of facial musculature has been reported in humans, chimpanzees (*Pan trodlodytes*), and rhesus macaques (*Macaca mulata*) (Burrows et al., 2009, Burrows et al., 2006, Diogo et al., 2009, Waller et al., 2008b). However, although the facial muscles of the gibbons and siamangs (*Hylobatids spp.* - which lie between the apes and rhesus macaques on the phylogenetic tree) show structural similarity with these species, they are less well developed (Burrows et al., 2011). Additionally, primates such as *Otolemur spp.* (bushbabies and galagos), have more complex facial musculature than previously reported (Burrows and Smith, 2003). Furthermore, new research systematically documenting the facial muscles and expressions of the domestic cat and dog has demonstrated extensive capacity and a surprising amount of similarity with humans and other primates (Caeiro, 2013, Ekman et al., 2002, Parr et al., 2010, Vick et al., 2007, Waller et al., 2013). Clearly, the structural mechanisms required to produce complex facial expressions are widespread, suggesting that either this is the ancestral form for mammals or that social and/or ecological factors have been influential selective pressures.

Determining whether similar traits in different species are the product of homologous or analogous processes is a difficult task, particularly for traits that leave no fossil records with which to reconstruct their evolutionary path. One of the most established ways to examine this is by studying the presence or absence (in if a trait is present, the form) of a trait in a wide range of taxa. If a range of animals with a common ancestor has a trait with the same similar basic layout then it might be assumed that the trait was inherited, and therefore homologous. Whereas if only a few distantly related animals share specific similar structures, yet diverge on many other traits, then it might be considered

that these represent a case of convergent evolution under common selective pressures. By considering what factors in the environment species share and how traits might function to provide individuals with advantages in response to environmental challenges, we can begin to consider what pressures may have promoted the evolution of such traits (Byrne, 1995). More detailed analysis involves studying whether structures involve the same precursor cells in embryos – a much more challenging task, which is not practical for widespread study (Diogo & Wood, 2012).

It has previously been suggested that facial expressions are more adaptive for diurnal terrestrial species, such as humans and horses, compared to arboreal species - as visual signals transmit better in an open environment (Chevalier-Skolnikoff, 1973, Dobson, 2009b, Kingdon, 2007). Indeed *Hylobatids* live in a densely forested environment whilst humans, chimpanzees, and rhesus macaques are plains dwellers. However, *Otolemur* are also arboreal and generally considered to be nocturnal, although there is evidence to suggest that they are active in the day and that vision and visual recognition of conspecifics is important to them (Bearder et al., 2006). Moreover, arboreality does not significantly correlate with facial mobility, potentially suggesting facial expressions are also adaptive in some forested environments (Dobson, 2009b, Waller and Micheletta, 2013).

Neuroanatomical and behavioural evidence demonstrates that instead, facial mobility is partially correlated with body size, where larger animals generally have higher facial mobility (although there are exceptions). Visual acuity is also positively correlated with body size and larger bodied species are better at processing the fine spatial details of visual stimuli than smaller species (Dobson, 2009a, Kiltie, 2000). When body size is controlled for, the key factor that correlates with the capacity to produce complex facial expressions is group size and more importantly, group complexity (Dobson, 2009b, Dobson, 2012). This corresponds with suggestions that facial expressions are rich sources of social information, and that they might have a key role in the management of social relationships and group cohesion, which is particularly important in complex social networks (Dobson, 2009a, Dobson, 2009b, Flack and de Waal, 2007, Parr, 2003, Waller and Dunbar, 2005).

However, to truly understand whether the production of complex facial expressions has communicative and adaptive value, we need to look not only at the producer of the signal but also the receiver. Capacity for and specificity of signal production does not actually establish whether the signal does convey specific information to observers: it simply describes the potential for doing so (Seyfarth and Cheney, 2003). The mechanisms underlying signal production and signal detection may be very different - potentially information extracted from a signal may be detrimental to the producer (Seyfarth and Cheney, 2003). Although work on animal facial expressions has generally received little attention, experimental work systematically examining what information receivers extract from signals and how this may be functionally relevant has been particularly neglected (Racca et al., 2012, Waller and Micheletta, 2013). In computer-based tasks, it has been shown that chimpanzees, rhesus macaques, and crested macaques (*Macaca nigra*) can match emotional facial expressions across different individuals. Furthermore, chimpanzees were able to match conspecific facial expressions to pictures representing a similar valence (e.g. a play face with highly preferred food items), suggesting that the chimpanzees had some, seemingly categorical representation of emotion (Micheletta et al., 2015, Parr and Heintz, 2009, Parr, 2001, Parr et al., 1998). This demonstrates the potential for gaining insights into animal cognitive representations using experimental paradigms, but these tasks do not indicate how the animals might use the information received from the facial expression. In addition to systematic work exploring the capacity to produce expressions, novel experimental paradigms that examine what information receivers extract from signals and how this may be functionally relevant will be necessary to fully understand the selective pressures driving the evolution of facial expressions.

Section summary

Facial expressions are a key means of communication in humans, but the extent to which this is true in other animals, particularly non-primates, is largely unknown. However, there is current uncertainty in the field regarding the evolutionary path to complex facial expression, which cannot be clarified without the ability to make direct comparisons across a range of varied taxa. Objective, anatomically based tools are providing new, systematic methods that can be used to make direct cross-species comparisons. These tools, combined with the development of naturalistic experimental paradigms that allow us to examine what information receivers extract from signals and

how this may be functionally relevant, will contribute to our knowledge on the evolution and function of facial expressions. This may also help us understand how some cognitive concepts are represented in animal minds.

Horses and primates are very distantly related in phylogenetic terms, so large differences between the species might be expected. Moreover, the common suggestion that only primates can produce complex facial expressions would lead to the prediction that the facial musculature of horses would be more rudimentary than that of primates, with fewer individual and distinctive muscles. On the other hand, horses have good visual acuity, an open environment that would not obscure facial expressions, and a need to maintain valuable relationships in complex societies. Therefore if socio-ecological factors have been influential then we might expect to see some convergences with primates. Similarly, if complex facial expressions are the result of retention of shared characteristics in a common ancestor, we would also expect to see similarities across diverse mammalian species. There have been reports of complex facial displays in horses, including a bared teeth display that may be analogous to the primate bared teeth display. However, there are currently no objective tools for measuring facial expressions in horses and nor has there been any experimental work investigating whether the production and perception of facial expressions has any adaptive value.

Sensitivity to the Attentional and Emotional States of Conspecifics

When encountering other individuals, there is a social problem to be solved: what is their likely behaviour and how may this affect you. This is particularly difficult in the case of dealing with unknown animals, where there is no previous knowledge on which to base judgements. How do you know whether to approach them or not? How do you ascertain whether these individuals constitute a threat to you and those connected with you? A sensitivity to their internal states would presumably be highly advantageous in these situations, and it has been argued that the study of mental attribution of others should be extended to all animals that live in complex social societies and display sophisticated social interactions (Emery, 2005). This also offers us a way to gain insights into how animals perceive the world. Below I outline the literature surrounding sensitivity to two key internal states: attention and emotion.

Attention

Attention is a bias of the perceptual resources to prioritise the information that is most relevant in the current situation (Nobre and Kastner, 2014). Being sensitive to the attention of others therefore could have adaptive advantages, for example alerting an animal to the presence of predators or a valuable food source in the environment (Goodwin, 2002). This can occur through relatively simple, bottom up mechanisms such as stimulus enhancement, where the action of an individual draws the attention of the receiver to the stimulus (Goodwin, 2002). Therefore while animals may be able to respond appropriately to a stimulus, it cannot be assumed that they understand what it means, or that they are able to attribute a visual perspective to the signaller. However, it is believed that humans at least have a more complex, top down, ability to respond to the attentional cues of others, where information is communicated about the internal state of the signaller and/or the object of attention (Aschersleben et al., 2008). Having this ability might enable animals to predict the behaviour of others and adjust their own behaviour accordingly. For example, many corvids store, or cache, food, but will alter their behaviour if being watched by a conspecific who may potentially raid the cache in future (e.g. the caching bird may cache behind a natural barrier, make false caches, or re-cache food once the watcher has left) (Bugnyar and Kotrschal, 2002, Bugnyar and Kotrschal, 2004, Dally et al., 2005, Emery et al., 2004, Wall, 1990). Sensitivity to another's attention is also widely accepted as at least a necessary (if not sufficient) requirement for intentional communication (Dennett, 1987, Schel et al., 2013). Consequently, there is a great deal of interest in discovering the capacity for attention attribution across species.

Humans are very good at following attention – the ability to follow someone else's gaze to objects outside your own visual field develops early on (before 18 months, Emery, 2000, Langton et al., 2000) and we can track the attention of numerous others in complex scenes (Von Grünau and Anston, 1995). We are sensitive to very subtle attentional cues, such as eye gaze, and will preferentially use eye gaze over head or body cues when following the gaze of another (Tomasello et al., 2007a). Humans have distinctive, forward facing and elongated eyes with a large amount of visible white sclera surrounding the darker iris. These adaptations are thought to afford us greater eye movement and to aid others in the detection of our eyes, in particular the direction of our gaze (Kobayashi and Kohshima, 2001, Kobayashi and Kohshima, 1997). The

prominent eyebrows of humans are another relatively unique feature, thought to enhance the salience of the eyes as a visual cue (Emery, 2000). These adaptations have generated particular interest in the evolutionary spread of the ability to use the eyes as a communicative cue.

As other primates, and particularly apes, have a close evolutionary relationship with humans and share some of the distinctive morphological features that humans have, it might be expected that they would also show advanced abilities in following the attention of others (Emery, 2000). Similarly, it has been suggested that as many non-primate animals have very differing eye morphology with laterally placed eyes, they cannot use cues from the eyes alone and must instead rely on head and body orientation (Emery, 2000, Kaminski et al., 2005). However, results from behavioural tests of primates show mixed abilities in this regard whilst other species, such as dogs, seem to perform surprisingly well. Few animals with laterally placed eyes have been tested on their ability to follow cues from the eyes alone, so it is difficult to evaluate these claims. Moreover, visual cues that humans lack have seemingly been overlooked in most experiments.

Sensitivity to others' attention in primates

There is strong evidence that most primates can respond appropriately to the attentional state of their audience. Much of this comes from tasks in which an animal has to communicate with an experimenter to get something (e.g. food) while the experimenter varies their attentive stance (e.g. turned towards or away from the recipient). All the great apes (chimpanzees, bonobos, *Pan paniscus*, orangutans, *Pongo spp.*, and gorillas, *Gorilla gorilla*) modify and increase their production of visual signals in appropriate ways depending on the recipients' attention (Genty et al., 2009, Hobaiter and Byrne, 2011, Leavens et al., 2010, Pika et al., 2005, Waller et al., 2015). There is also observational and experimental evidence of appropriately modified behaviour in response the attentional state of an observer, and even the manipulation of another's attention, for deceptive purposes in a number of primates (Hare et al., 2006, Whiten and Byrne, 1988). It could be argued that a social audience can trigger communicative behaviours through several low-level mechanisms including increased arousal, reflexive responses to the presence of another, and social interaction that is removed when the other party is not attending (Waller et al., 2015). However, there is also evidence that

visual signals can be used flexibly and intentionally in these situations, suggestive of higher order cognitive processes that go beyond the capacity of low-level mechanisms. Moreover, sometimes the incidents of tactical deception reported are so sophisticated that it is difficult to conceive a more simplistic explanation that does not require the attribution of attention and at least a rudimentary representation of another's internal states (Whiten and Byrne, 1989).

Other research has studied attention following in object choice tasks (where a model displays attention to one of a number of objects and the response of the subject is monitored), or competition tasks (where the animal can chose to compete with another based on what information the other has previously seen) and has attempted to identify whether some signals are more salient than others. The results of these investigations have offered conflicting evidence regarding the specific attentional cues that primates are sensitive to. There is evidence that chimpanzees can follow a human experimenter's attention, as signaled by body orientation, head orientation, and eye gaze when the cues are given simultaneously and individually (Bulloch et al., 2008, Povinelli and Eddy, 1996a, Povinelli and Eddy, 1997). Bonobos, orangutans, and gorillas can also follow human gaze when given as a combination of head and eye orientation. All great apes seem to be aware of the effect of barriers on another's vision, adjusting their behaviour depending on what another (human and conspecific) can see or has seen. They will also reorient themselves to account for a human signal giver's perspective, although orangutans – the less social ape – were less skilled (Bräuer et al., 2005, Hare et al., 2006, Hare et al., 2001, Liebal et al., 2004, Okamoto-Barth et al., 2007, Peignot and Anderson, 1999, Tomasello et al., 2007b). These results suggest that chimpanzees are sensitive to the attention of another from eye gaze alone, and that all great apes have some knowledge of what another can see and some ability to perspective take.

However other work has disputed this and claimed that while chimpanzees can follow attention they cannot discriminate what another has seen, use the attentional state of another to learn about objects in the world, or understand the internal state of the cue giver (Hare et al., 2001, Povinelli and Eddy, 1996b, Povinelli and Eddy, 1997). Some research, suggests that although all great apes can assess human attention based on head and body orientation in a begging task, and may even show some knowledge about the ability for the experimenter to hand over food based on body orientation, they are

generally unable to use cues from the eyes alone (Kaminski et al., 2004, Peignot and Anderson, 1999, Tempelmann et al., 2011). Similarly, young chimpanzees can discriminate between people who had their whole head covered or not but not between people with their eyes open or closed (Povinelli et al., 1996, Reaux et al., 1999).

Results from other primates are similarly mixed. Ring-tailed, brown, and black lemurs (*Lemur catta, Eulemur fulvus, Eulemur macaco*) can co-orient with their conspecifics and use this behaviour to find hidden food, and ring-tails show some sensitivity to human visual attention (Bray et al., 2014, Jolly, 2004, Ruiz et al., 2009, Shepherd and Platt, 2008). Tomasello et al. (1998) found that sooty mangabeys (*Cercocebus atys*), rhesus macaques, stumptailed macaques (*Macaca arctoides*), and pigtailed macaques (*Macaca leonina*) could follow the attentional cues of conspecifics when semi-free ranging in a large outdoor enclosure, and the stumptailed macaques even outperformed chimpanzees in this task. Additionally, gaze following in macaques is influenced by relevant social relationships - dominance in despotic rhesus macaques, friendship in egalitarian crested macaques - and there is neurological evidence of selective cells in the macaque brain that are sensitive to the head orientation and isolated eye gaze cues of conspecifics (Perrett et al., 1985, Micheletta and Waller, 2012, Shepherd et al., 2006).

However, Itakura (1996) reported that of a range of eleven species of prosimians, monkeys and apes, only the chimpanzees and orangutans could follow a human experimenter's gaze, even when multiple cues were available (eyes, head and pointing in a corresponding direction). Other studies have found capuchin monkeys (*Cebinae spp.*) and rhesus macaques cannot use human gaze or pointing to locate food spontaneously, and although a few individuals could learn to use these cues after extensive training only one monkey could then transfer these skills to a novel experimenter (Anderson et al., 1996a, Anderson et al., 1995, Vick and Anderson, 2000). Furthermore, baboons (*Papio spp.*) and long tailed macaques (*Macaca fascicularis*) do not use the attentional cues of others to find hidden food, even though they can co-orient with conspecifics and baboons adjust their visual and auditory intentional gestures to the visual attention of others (Bourjade et al., 2014, Fagot and Deruelle, 2002, Vick et al., 2006).

Sensitivity to others' attention in non-primates

Dogs show remarkable sensitivity to human attention and the ability to use this information to inform them about their environment (Hare and Tomasello, 2005, Miklosi and Soproni, 2006). Dogs can distinguish between effective and ineffective barriers to a handler's sight and will only steal food if a person cannot observe them (Bräuer et al., 2004, Kaminski et al., 2013). Dogs are more likely to obey commands when humans are looking at them and have their eyes open, and only use gaze if it is specifically directed at a target, suggesting that they may have some form of appreciation of the communicative intent underlying the cue (Call et al., 2003, Soproni et al., 2001). Dogs are also sensitive to the attentional state of their conspecifics, although less work has explored this compared to studies investigating their sensitivity to human attention (Hare and Tomasello, 1999, Horowitz, 2009). Interestingly, wolves (*Canis lupus*) do not show these sophisticated skills in responding to human attention and so it has been suggested that dogs' exceptional sensitivity is due to domestication, and may represent a case of convergent evolution (Hare and Tomasello, 2005, Hare et al., 2002, Miklosi and Soproni, 2006, Virányi et al., 2008).

As a result of these findings, there has been interest in studying the sensitivity of other domestic species to human attention. The famous case of Clever Hans demonstrated that domestic horses can be highly sensitive to subtle human cues, and experimental work has since gone on to demonstrate that while adult horses are skilled at responding appropriately to human attention from subtle cues, they are less adept at using human communicative cues in object choice tasks (Krueger et al., 2011, Maros et al., 2008, McKinley and Sambrook, 2000, Proops and McComb, 2010, Proops et al., 2010). Additionally, horses' ability to determine whether humans are attending to them using subtle body cues appears to require significant experience to fully develop, and so horses differ in this from dogs who show advanced skills early on (Hare and Tomasello, 2005, Proops et al., 2013). In a parallel to dogs, no research has investigated the ability of horses to determine conspecific attention and so it is difficult establish whether the differences seen in these domestic animals reflect their inherent communication systems or differing levels of experience with humans and/or selection by humans for certain characteristics.

Interestingly, some animals that have never been domesticated but interact with humans

(e.g. bottlenose dolphins, *Tursiops truncatus*, and African elephants) respond appropriately to human attentional states and understand the communicative intent of some cues (Pack and Herman, 2007, Smet and Byrne, 2014a, Smet and Byrne, 2014b, Smet and Byrne, 2013, Tschudin et al., 2001). The ability to respond appropriately to human attention has also been seen in black iguanas (*Ctenosaurua similis*), tufted titmice (*Baeolophus bicolor*), Carolina anole lizards (*Anolis carolinensis*), and hog nosed snakes (*Heterodon platirhinos*), and it appears that in these cases visibility of the eyes may be a critical cue (Burger et al., 1992, Burghardt and Greene, 1988, Freeberg et al., 2014, Hennig, 1977). Naturalistic paradigms have also demonstrated that tortoises (*Geochelone carbonaria*), domestic goats (*Capra hircus*), and domestic pigs (*Sus scrofa*) respond to the attentional states of conspecifics (Held et al., 2001, Kaminski et al., 2005, Wilkinson et al., 2010), and as discussed earlier, corvids show advanced abilities to determine and manipulate the attention of conspecifics, and may even use their beak to point at their object of attention (Bugnyar and Kotrschal, 2004, Bugnyar and Kotrschal, 2002, Dally et al., 2005, Emery et al., 2004, Kaplan, 2011, Wall, 1990).

Taken together, this research suggests that a wide range of species are sensitive to the attentional state of others; however, only a few species are consistently found to be able to use this ability to gain information about their environment. This presents us with an evolutionary paradox – why have these skills if they have no functional relevance? Potentially, there could be other adaptive advantages that sensitivity to attention confers which we are currently unaware of, or the ability to follow attention is a by-product of another skill. However, there may also be some more obvious explanations in terms of the methods used to carry out these experiments.

Studying attention following

Human influence

Many of the experiments studying attention following use human demonstrators and so are specifically investigating the sensitivity of animals to human attention. This can be influenced by the amount of experience that animals have with humans and may therefore be responsible for some of the unusual and seemingly contradictory results seen, particularly in chimpanzees (Bulloch et al., 2008, Emery, 2000, Kaminski et al., 2004). Whilst investigating sensitivity to human attention is a very relevant question for

understanding the effects of domestication and co-existence with humans, to answer questions about sensitivity to attention as a general social mechanism, knowledge of conspecific communication systems is crucial. Moreover, even when investigating intraspecific interactions there has been a general bias to studying cues that humans use: head orientation, body orientation, and eye gaze. This overlooks the fact that many animals have a very different morphology to humans and might have other means of signalling. Consequently, if we want to truly investigate communication in a wide range of species, we need to also consider potential cues to attention that we do not have.

Artificial paradigms

The experimental set up and procedure seems to be critical in determining the results of attention following tasks. Performance is influenced by the willingness of participants to engage in the tasks and co-orient with the model (Itakura, 1996, Ruiz et al., 2009). Chimpanzees quickly habituate and stop producing gaze-following responses if exposed to a human repeatedly looking to a location when there is no object present (Tomasello et al., 2001). Chimpanzees can also distinguish between intentional and unintentional human actions and may have sophisticated understanding of the physical set up of experiments and the realism of the task presented (Call and Tomasello, 1998, Call et al., 1998). Minor changes in experimental protocols, for example the position of the subject relative to the experimenter, influence the performance of apes in object choice tasks; and chimpanzees show advanced abilities in competitive contexts compared to co-operative tasks, perhaps because competitive tasks better represent the nature of their interactions with conspecifics (Barth et al., 2005, Hare, 2001, Mulcahy and Call, 2009). Consequently, the ability to present naturalistic paradigms that engage subjects and examine spontaneous interactions is crucial for obtaining valid results.

In line with this, studies using naturalistic gaze following paradigms with conspecific models have produced more consistent results. However, within these it is difficult to control the signals produced by the model and establish exactly which are informative. Little detail is given on the range of cues that the model produces in such experiments and gaze is often used as a general term encompassing head orientation, eye direction, facial expression, and any other potential indicators that may be present. Thus, it is difficult to test and evaluate theories about the ability to use particular cues and how

these signals may be represented or prioritised by the receiver. For sensitivity to the attention of others to be adaptive it should be partly flexible, as there will be situations where the face or the body is occluded (Emery, 2000). Whilst this is difficult to achieve in a naturalistic situation, current methods to investigate this in more controlled paradigms include presenting signals (such as eye gaze) in isolation or simultaneously but with conflicting directions (Rosati and Hare, 2009). Yet, potentially vital aspects of an attentive signal are overlooked in these controlled experiments, which might have confounding effects on the results. Facial expression is known to influence the perception of attentional cues and gaze following is enhanced when gaze is given with a facial expression rather than a neutral face (Bindemann et al., 2008, Lobmaier et al., 2008, Teufel et al., 2010). Furthermore, although conflicting signals are quite common (we do not always gaze straight ahead of us) when ambiguous cues are encountered they are interpreted according to their context (Chun, 2000).

Section summary

Being sensitive to the attention of others is a fundamental skill that is likely to have significant adaptive advantages. Consequently, the advanced abilities of humans to respond appropriately to the attentional states of others and act on this information are unlikely to be completely unique. However, studies investigating the capacity of non-human animals to follow attention and use this to enhance their environmental knowledge have reported conflicting results. This is likely due to methodological issues, such as use of a human experimenter and unrealistic paradigms. Moreover, current work has overlooked the potential for other species to be using cues to attention that humans do not have. To progress, we now need to develop innovative, naturalistic paradigms that investigate a broader range of potential cues to attention.

Horses show striking sensitivity to the attentional state of humans, yet they do not seem to be able to use the information present in human attentional cues very effectively in object choice tasks. However, no previously published studies have investigated the sensitivity of horses to the attention of their conspecifics. As horses have laterally placed eyes it may be predicted that they cannot use information from the eye area alone, but must be relying on other cues. Notably, horses, along with many other animals, have large mobile ears that could act as visual signals, but this has never been

tested. Moreover, in the domestic environment horses often have parts of their body, including areas of their faces, obscured by equipment such as blinkers. This provides the unusual opportunity to isolate signals in a realistic and familiar way.

I now turn to the next social skill that this thesis investigates, the sensitivity of animals to emotional states in others.

Emotion

Although working definitions of emotions vary, there is a consensus that emotions are multi-faceted response tendencies, which are directly elicited by a stimulus and promote adaptive behaviour in that situation (Adolphs, 2010, Fredrickson, 2004, Frijda, 2009, Frijda, 1986, de Waal, 2011). These responses are intense, short-lived and are directly attributable to an event; distinguishing emotions from moods, which are longer term affective states derived from a combination of experiences (Adolphs, 2010, Mendl et al., 2010). Emotions consist of both physiological and behavioural changes, for example, fear is by marked by increased respiration, blood pressure, and heart rate, redistribution of blood from the digestive system to muscles, heart, and brain, and release of sugar from liver reserves, which are physiological preparations for increased vigilance and likely escape behaviour (Adolphs, 2010, Barrett, 2006, Cannon, 1914, Mendl et al., 2010). The concept, existence and expression of emotion in non-human (and even human) animals are controversial issues that have been debated for well over a century (Darwin, 1999, Mendl and Paul, 2004, Panksepp, 2005a, de Waal, 2011). However, it is now more widely accepted that animals are sentient beings who experience emotional states, and there is growing interest in what these states are, how they are represented cognitively and neurologically, and how they are expressed (Panksepp, 2005b, Panksepp, 2005a, Council of the European Union, 1997).

Categorical classifications of emotion, which divide emotions into a number of discrete experiences, have received much support (Darwin, 1999, Ekman et al., 1969, Ekman, 1999, Izard, 1994, Panksepp, 1998, Panksepp, 2005a). It has been argued that, at least in mammals, there are separate ‘basic’ emotional systems (e.g. fear, rage, panic, play) rooted in the neural circuitry of particular brain areas, serving specific adaptive functions, and representing the fundamental building blocks of all emotional reactions

(Ekman, 1999, Panksepp, 2005a, Panksepp, 1998). This has presented some interesting evidence demonstrating commonalities in the neurological circuits responsible for certain behavioural responses of diverse mammalian species, e.g. rats and humans. However other research has argued for a more dimensional approach, which characterises emotions in terms of core affective characteristics, such as valence (positive or negative) and arousal (high or low) (Briefer et al., 2015, Briefer, 2012, Russell, 2003). Ultimately, it seems an integrative approach that incorporates both these models fits the available scientific evidence best, but further work that enhances our understanding of the cognitive and neurological representation of emotion across a range of species is needed (Mendl et al., 2010). In particular, there have been calls to shift the field away from the focus on whether emotions that humans feel are present in other animals, and towards questions about whether there are shared emotional mechanisms and functions across species (LeDoux, 2012).

One widely accepted aspect of emotions is that they motivate the animal to act in an adaptive way. Negative emotions are suggested to initiate a specific set of actions, thoughts, and behaviours, which can be enacted quickly and are highly applicable in threatening situations. Conversely, positive emotions are thought to facilitate approach behaviour in safe environments, which might create opportunities for environmental exploration, affiliative social contact, mating, and dispersal (Cacioppo and Gardner, 1999, Davidson et al., 2000, Fredrickson, 2004, Frijda, 2009). More broadly, it has been suggested that positive emotions promote a wide range of exploratory thoughts and actions, which in the long term lead to an extension of an individual's behavioural repertoire and not only promote but also build individual resilience (Fredrickson, 2004). This contrasts with the direct and immediate adaptive benefits that negative emotions elicit in situations that threaten survival, and takes advantage of the fact that positive emotions tend to happen in non-life threatening situations, in which animals have the time to explore and broaden their behavioural repertoire and personal resources.

Measuring emotional experience

As animals lack the ability to provide us with verbal report of emotions, reliably establishing their affective states is a challenging task. However, the reported subjective experiences of affect in humans are accompanied by neural, behavioural, physiological

and cognitive changes, such as alterations in brain activity, facial expressions, heart rate and attention to threat. These changes can be measured objectively and are widely considered to provide good evidence for the existence of affective state (Désiré et al., 2002, Ekman, 1999, Ekman et al., 1983, Mendl et al., 2010, Veissier et al., 2009).

Global behavioural measures

Negative emotions are generally characterised by avoidance, freezing, aggressive, and uncooperative behaviours, along with an increase in self-directed or abnormal behaviours, such as excessive self-grooming, stereotypies, and excessive defecation/urination (Désiré et al., 2002, Forkman et al., 2007, Fredrickson, 2004, Piva et al., 2008). Conversely the expression of anticipatory behaviour, play, exploratory behaviours (both towards other animals and the physical environment), and co-operative behaviours are accepted as indices of positive affect (Boissy et al., 2007, Burgdorf and Panksepp, 2006, Fredrickson, 2004, Piva et al., 2008). There are some limitations to using behaviour alone as an indicator of affective state, for example increased movement is considered as a positive measure but may also be an indicator of frustration. The most promising methods of study have involved situations that present an animal with stimuli and allow the animal to express its own preference, for example in approach-avoidance paradigms. Excellent examples of this are to be found in cognitive bias tests, in which animals are given ambiguous stimuli and their affect is measured by whether they make an optimistic (positive) or pessimistic (negative) judgement in a forced choice task with two options that have previously been conditioned as positive or negative. As information processing is biased by emotional state, if ambiguous events are interpreted negatively or positively this is thought to reflect the underlying emotional state of an individual (Harding et al., 2004). This approach has provided unique insights into the perception and experience of animals where they can be trained to do these tests.

Vocalisations

The production of vocalisations is influenced by the emotional state of the signaler, which causes changes in the muscular tension and action of the vocal apparatus that impact on vocal parameters (Briefer, 2012). Consequently they are a promising indicator of emotional states, and several types of different vocalizations have been

shown to indicate positive or negative emotional valence. In humans, the affect from some non-verbal vocalisations can be recognised cross-culturally (Sauter et al., 2010). Moreover, the link between some vocalisations and specific brain circuits responsible for emotions has been established in some non-human species (e.g. rats, Burgdorf and Panksepp, 2006).

There is evidence for vocalisations indicative of negative affect in a number of species. Yet, these calls often vary between species and so as indicators of emotional affect they need to be considered in species-specific contexts. Grey mouse lemurs (*Microcebus murinus*) produce whistles when isolated (Scheumann et al., 2007); rats (*Rattus norvegicus*) produce an ultrasonic vocalisation in a variety of negative contexts (Knutson et al., 2002); domestic cats produce growls and hisses when threatened (Yeon et al., 2011); chimpanzees scream when victims of aggression or when experiencing social frustration (Slocombe et al., 2009); pigs also give screams and squeals during aversive events (Reimert et al., 2013); and horses groan when in pain, and squeal when in agnostic encounters (McDonnell, 2003, Rubenstein and Hack, 1992, Waring, 2003). Some of these vocalisations may also give additional information that is relevant to the situation, demonstrating their potential communicative value. Horse squeals given in agonistic contexts also advertise the fitness of the caller (Rubenstein and Hack, 1992); chimpanzees' screams contain information about the level of aggression being received (Slocombe et al., 2009); and meerkat (*Suricata suricatta*) alarm calls contain both motivational information about the urgency of the threat and referential information about the specific predator type (Manser et al., 2002)

In relation to positive affect, all the great apes and some other primate species (such as Barbary macaques, *Macaca sylvanus*) utter laughter-like sounds during play. There is also strong evidence for a laughter-like vocalisation in rats (Darwin, 1999, Davila Ross et al., 2009, Kipper and Todt, 2002, Knutson et al., 2002, Panksepp and Burgdorf, 2003, De Waal, 1988). In addition, a wide range of animals also utter breathy, pulsated vocalisations in positive, although non-playful, contexts, e.g. the nicker of the horse (Waring, 2003), purring in felids (*Felidae spp.*) (Peters, 2002), and a purring-like vocalisation in other mammals, e.g. grey mouse lemurs (Scheumann et al., 2007). Pigs and dogs utter barks in positive situations (Reimert et al., 2013, Yin and McCowan, 2004) and so it seems likely that as with negative vocalisations, there may be a range of

positive vocalisations given in species specific ways. However, as with many measures of affect there is less work documenting the vocalisations associated with positive situations compared to negative situations, and the level of information that these calls contain (Boissy et al., 2007, Briefer, 2012)

Facial expressions

Humans produce a wide variety of facial expressions in emotional situations, which are elicited even when stimuli exposure is subliminal (Dimberg et al., 2000). There is some degree of universality in the facial expressions of human emotions as some expressions can be recognised cross-culturally, and elements of this may extend to non-human animals (Darwin, 1999, Ekman and Friesen, 1971, Ekman et al., 1969, Elfenbein and Ambady, 2003). Therefore facial expressions may be a good indicator of emotional experiences in animals, if we can measure them reliably.

Humans, sheep (*Ovis aries*), and horses widen their eye aperture in fear responses (Reefmann et al., 2009b, Waller et al., 2008a, Waring, 2003). Humans, horses, and cows (*Bos taurus*) also increase the amount of visible white sclera in fearful and stressful situations demonstrating that it is not a visual signal that is unique to humans, although it may be exaggerated in humans (Sandem et al., 2006, Sandem and Braastad, 2005, Sandem et al., 2004, Sandem et al., 2002, Whalen et al., 2004, pers. obs.) The eyebrows are raised in fear and sadness in humans, and the skin above the eyes is also raised in negative situations for horses (Waller et al., 2008a, S. Hintze, in prep.). In contrast to the general increasing of the eye area in fearful/sadness situations, aggressive and painful situations are characterised by a tightening of the eyelids and reduction of the eye aperture in humans, mice (*Mus musculus*), rabbits (*Oryctolagus cuniculus*), rats, and horses (Dalla Costa et al., 2014, Keating et al., 2012, Langford et al., 2010, Sotocinal et al., 2011, Waller et al., 2008a). These same animals have also been shown to present a lifting and wrinkling the skin around the nose when in pain, along with a retracted/raised lip in humans, horses, and sheep (Jochle et al., 1989, Molony et al., 2002, Williams, 2002). The retracted lip is seen in a number of primates (often termed a fear grin, fear grimace, or bared teeth submissive gesture) in response to fearful situations, and particularly in submissive situations, and has also been observed in horses (van Hooff, 1972, Parr et al., 2007, De Waal, 2003, Waller and Dunbar, 2005,

Waring, 2003; Figure 1). Pursed lips and a raised chin are associated with anger in humans, chimpanzees, and horses, and may also be associated with pain in the horse (Dalla Costa et al., 2014, Parr et al., 2005, Waller et al., 2008a, pers. obs., K McComb, pers. com.).

Animals with control over the movement of their vibrissae (whiskers) may incorporate this into their facial displays, and forward bunching of the whiskers is seen in rats and rabbits when in pain (with unspecified whisker movement associated with pain in mice) (Keating et al., 2012, Langford et al., 2010, Sotocinal et al., 2011). Additionally, animals with large mobile ears are commonly reported to hold them in predictable patterns depending on their emotional valence. Holding the ears pointing and flattened backwards is generally associated with aggression, for example in macaques, horses, and mice (Andrew, 1963b, Defensor et al., 2012, Waring, 2003). Holding the ears asymmetrically seems to be associated with negative valence in a number of species, e.g. horses, sheep, rats, and mice, and holding the ears low is associated with pain in horses and mice (Boissy et al., 2011, Gleerup et al., 2015, Langford et al., 2010, Reefmann et al., 2009a, Sotocinal et al., 2011).

Figure 1. Snapping, or the bared teeth submissive gesture.

There is a distinctive ‘play face’ featuring an open mouth with relaxed lips that often cover the teeth, which is seen in a range of animals including humans and numerous other primates, polecats (*Mustelidae spp.*), canids (*Canidae spp.*), and horses, (Fox, 1970, Pellis and Pellis, 1997, Poole, 1978, Reed and Brown, 2000, pers. obs, Figure 2).

Although a reduction in the eye aperture is associated with some negative emotions, it is also associated with positive affect in humans, cats, and canids (Ekman et al., 1990, Fox, 1970, Tabor, 2003). Therefore there must be something quantitatively different about the eye narrowing, or about the rest of the face that differentiates eye narrowing in these affective situations. Indeed in humans, raising the infraorbital area creates the eye narrowing in positive situations whereas eye narrowing in anger is due to contraction of the eyelids. Whether similar differences exist in other species is not yet known. In animals with mobile ears, holding them in a forward position or in a relaxed, neutral, state is thought to indicate positive affect (Boissy et al., 2011, Proctor and Carder, 2014).

However, whilst there has been a strong drive to identify facial expressions associated with negative emotional states in a range of species, e.g. (Dalla Costa et al., 2014, Keating et al., 2012, Langford et al., 2010, Sotocinal et al., 2011), the systematic study of positive facial expressions has largely been limited to primates, e.g. (Burrows et al., 2009, Parr, 2003, Parr et al., 2005), and it has been proposed that most animals, and in particular non-primates, are not able to display positive emotions in their facial expressions (Boissy et al., 2011, Tate et al., 2006).

Figure 2. Open mouth play face displayed in the horse on the left of the image.

Physiological measures

Changes in emotional state create physiological changes, such as altered levels of glucocorticoids and alterations in heart functioning (Boissy et al., 2007, von Borell et al., 2007, Panksepp, 1998). Cortisol is this most commonly measured glucocorticoid, with increased cortisol levels associated with stress and negative affect (Buchanan et al., 1999, Cheney and Seyfarth, 2008, Glenk et al., 2013, Sapolsky, 2007). Cortisol has the advantage that it is can be collected remotely (through fecal samples) and so can give us an insight into the stress levels of animals that we cannot get close to, although the analysis can be time consuming and expensive. With animals (we can approach) heart functioning is an accessible measure that can be recorded and analysed effectively with commonly available equipment. Average heart rate is a very effective measure of emotional affect, and increased heart rate is associated with behavioural changes of fear whilst decreased heart rate is associated with positive experiences, such as grooming at a preferred site (Feh and de Maziéres, 1993, Leiner and Fendt, 2011). The assessment of heart rate variability is becoming increasingly popular, as increased heart rate variability is assumed to represent a positive experience and a decrease in variability a negative experience (von Borell et al., 2007). However to gain accurate measurements, heart rate variability needs to be measured over a longer period of time than basic heart rate, and so is not suitable for all research questions (von Borell et al., 2007).

Multifaceted approaches

Whilst the behavioural and physiological measures reviewed above all provide promising ways to gain insights into the affective experience of animals, none are without their limitations. Global behavioural measures can be non-specific and context dependent, illustrated by the fact that increased activity is often listed as a measure of both positive and negative affect (e.g. Boissy et al., 2007, McGreevy et al., 1995). Situations likely to induce different affective states may evoke similar physiological responses that could simply be indicative of arousal. Heart rate, for example, may rise in both fear inducing situations and in anticipation of rewarding stimuli (Baldock and Sibly, 1990). Similarly, there may be some adaptive benefits associated with the ability to mask emotions, such as pain or discomfort in order to minimise the risk of predation (Mayer, 2007, O'Callaghan, 2002) - therefore outward behavioural signs may not

necessarily reflect the emotional state of the animal. Consequently, there is a consensus that physiological measures coupled with observations of behaviour represents one of the most effective ways to accurately determine the animal's affective state (Baldock and Sibly, 1990, Barrett et al., 2007, Barrett, 2006, Reefmann et al., 2009b).

Emotion attribution

Although there are signals given in a wide range of species that are reliably associated with emotional contexts, in non-human animals there have been surprisingly few experiments that investigate the perception of these signals by conspecifics, what information these signals contain, and how this information is meaningful (Murphy et al., 2014). Humans have a cross-modal ability to recognise others' emotional states, deficits of which can lead to social and functional impairment (Ruffman et al., 2008). Humans can recognise affective facial expressions and non-verbal emotional vocalisations, and can do so cross-culturally for some emotions (Ekman et al., 1969, Elfenbein and Ambady, 2002, Sauter et al., 2010). However, in situations of very high arousal humans cannot distinguish emotional valence from the face alone, and instead rely on cues from the body posture (Aviezer et al., 2012). This evidence supports an integrative model of cognitive emotional representation (Mendl et al., 2010), and demonstrates the potential of experimental paradigms to help us understand cognitive processes and representation. Additionally, work studying human perception of emotion has demonstrated that seeing another human having an emotional experience automatically activates behavioural and neurological mechanisms that allow us to represent the emotion internally (Lee et al., 2006, Likowski et al., 2012, Warren et al., 2006, de Waal, 2011). This provides valuable insights into the physiological and neurological processes underpinning the transfer of information.

In non-human animals, playback experiments have demonstrated that in a range of animals urgency/motivation is reliably encoded in alarm calls and distress vocalisations, which are recognised by conspecifics and serve to inform the receiver's behaviour, e.g. enacting appropriate predator responses or recruitment of help in conflict (Manser et al., 2002, Slocombe et al., 2009, Slocombe and Zuberbühler, 2007). Reaction to these calls is also influenced by social relationships in some species (Micheletta et al., 2012). A similarly adaptive effect has also been demonstrated in response to hearing positive

vocalisations in rats, who approach playbacks of conspecific ultrasonic vocalisations associated with positive affect (Wohr and Schwarting, 2007). Rats can also recognise when conspecifics are in distress or pain from whole body visual cues, and will act to release the distressed conspecific or avoid the area that the pained conspecific is in (Bartal et al., 2011, Nakashima et al., 2015, Sato et al., 2015). These experiments demonstrate the ability for animals to use the information in the emotional expression of others to inform them about environment, and are consistent with theories on the function of emotion.

However, experiments investigating the perception of facial expressions, a key behavioural indicator of emotion, have tended to focus on the ability to discriminate between expressions whilst overlooking how the information gained from these cues might be adaptive. Generally, the perception of emotional facial expressions in non-human animals is examined through computer-based tasks, which must be interpreted with some caution because the participating animals tend to be extensively trained on visual matching of stimuli. These tasks have given us some insights into the representation of emotion in non-human animals, suggesting that chimpanzees represent emotions categorically and that chimpanzees and dogs may have specialised cognitive systems for processing facial expressions of negative valence based in the right hemisphere (Parr, 2003, Parr and Hopkins, 2001, Racca et al., 2012). However, such experiments lack the ability to give us insight into how the information that receivers gain from the signals may help them to function in their world.

Section summary

It is becoming more widely accepted that animals have emotions, although the exact nature of how animal emotions are represented, expressed, and perceived by others is still unclear. Although animals cannot provide verbal feedback on their affective experiences we can ask them to provide feedback in other ways, such as making a choice or measuring their behavioural and physiological responses to stimuli. Studying the reaction of animals to conspecifics displaying emotional expressions can therefore provide us with insights into how they judge that emotional experience, as well as how it is processed and represented cognitively. Furthermore, we can investigate how the information in these expressions may have a communicative value that is adaptive.

Research in some modalities has offered evidence to support this, while research investigating facial expressions in non-human animals has yet to develop novel experimental paradigms that would allow us to explore the perception of facial expressions with regard to its adaptive value.

Whilst horses are an ideal study species in which to investigate the evolution of social cognition and communication, due to their popular domestic status there is also a great deal of interest in how they experience emotions, how they express those experiences, and what they recognise of emotional experience in others. Therefore, understanding the emotional lives of horses may not only inform scientific endeavour but also have significant implications for horse management and welfare.

The Study Species: The Domestic Horse

Horses are members of the family *Equidae*, in the order *Perissodactyla* (odd-toed ungulates). *Equidae* only contains one extant genus, *Equus*, which is comprised of nine species of equid: the domestic donkey (*E. asinus*); three species of ass (*E. africanus, E. hemionus, E. kiang*); three species of zebra (*E. quagga, E. zebra, E. grevyi*); and two species of horse, the domestic horse (*E. caballus*) and the Przewalski's horse (*E. ferus przewalskii*). There has been some debate about whether the domestic horse and the Przewalski's horse should be considered as subspecies of *E. ferus* as they can interbreed to produce (infertile) offspring, but recent genetic analysis indicates that they are distinct species and probably diverged around 43 thousand years ago (Orlando et al., 2013, Waring, 2003).

The Equus lineage that gave rise to our current equids originated between four and four and a half million years ago (Orlando et al., 2013). By the end of the Pleistocene era (approx. 2.5 million – 12,000 years ago) wild horses (*E. ferus*) roamed worldwide, with fossil remains identified on every continent except Antarctica and Australia. The domestication of horses is estimated to have begun in the western Eurasian steppe (Ukraine and Kazakhstan) around 5000 years ago and they have since thrived as a domestic species (Ludwig et al., 2009, Outram et al., 2009, Warmuth et al., 2012). Conversely, by the middle of the twentieth century *E. ferus* had become extinct in the wild, largely due to human pressures (Clutton-Brock, 1999). Populations of 'wild'

horses today are typically feral domestic horses or the Przewalski's horse (Waring, 2003). These populations can give us an insight into the natural behaviour of the domestic horse when not subjected to domestic pressures, and much of the behaviour observed in feral horses is also displayed in free-ranging domestic populations (Crowell-Davis, 1993, Waran, 1997, Christensen et al., 2002)[1].

Horse lifestyle

Horses are long-lived social mammals. While in domestic conditions horses may live for more than 40 years, in the wild average lifespan is lower and varies depending on ecology and group composition; small and unstable groups have the lowest life expectancy (Waring, 2003). Within an hour of birth, foals can generally stand, show binocular orientation, seek care giving behaviour, and display defence behaviours. Some foals may already be ingesting, vocalising, and defecating. Yet despite this apparent independence, foals will stay with their mother for at least a year and often longer if the mother does not give birth to another foal (Waring, 2003). Care of the foal is provided by the mother although foals will interact with, and may receive protection from, other group members (Feist and McCullough, 1976). The social connections of juveniles early in life is highly correlated with the social connections of the parents, particularly the mother, and mares are most likely to let previous offspring and preferred social associates interact with the foal (Sundaresan et al., 2007, Tyler, 1972, Weeks et al., 2000). Foals will typically remain with their natal groups for between two and four years when individuals of both sexes will disperse, although males are more likely to disperse than females (Tyler, 1972, Waring, 2003).

Horses typically live in a society comprising of several small groups or 'bands' that share space and resources, and to which membership stays relatively stable over time. These bands may contain more than twenty individuals (although more typically less than ten), and comprise of a stallion, multiple females, and their offspring; multiple males and females plus offspring; or entirely young males (bachelor bands). Bands are not usually territorial, but have large ranges that overlap with neighbouring bands so

[1] Feral horses are free-ranging populations that began from escaped domestic stock, but now roam and breed with limited human intervention. Free-ranging domestic populations live in large herds under naturalistic conditions, but still have regular contact with humans for interaction and activities such as riding and grooming.

horses regularly come into contact with many other conspecifics as they move and feed, and inter-band dominance indicates that within the larger herd established social relationships exist (Rubenstein, 1981, Waring, 2003). In particular, after dispersal foals may still interact with their natal groups, and stallions are significantly more likely to play with their dispersed sons than any other male (Berger, 1986). Interestingly, this social organisation is not typical of all equids, and some (e.g. Grevy's zebra, *E. gevyi,* and the Przewalski's horse) do not form fixed groups or strong social bonds (Klingel, 1974).

The preference for stable, prolonged associations between individuals is strong in horses, even those in very small, unnatural domestic groups - mismanagement of the need for these social relationships seems to be the cause of many behavioural issues in domestic horses (Klingel, 1975, Lee et al., 2011). Social play begins from around 3 weeks, and although it occurs most often in young horses, adult horses will continue to play throughout their lifespan. Play will occur with members of the band and with members of other groups (Waring, 2003). Groups revolve around the adult females, and long-lasting social bonds between female horses are common. Stallions have a patriarchal role and largely provide defense for the group, whereas dominant mares are more likely to be responsible for determining group decisions, such as movement (although horses are fairly egalitarian in making group decisions, Krueger et al., 2014, Waring, 2003). In this sense horse societies have been described as matriarchal, and females have been demonstrated to show more advanced social abilities than males in some domains (Goodwin, 2002, Proops and McComb, 2010).

Social skills

Horses are able to recognise a large number of familiar conspecifics and humans, and can integrate cross modal identity cues (Lampe and Andre, 2012, Proops and McComb, 2012, Proops et al., 2009). Horses are also able to learn to discriminate between human faces in photographs, and then spontaneously transfer this identity knowledge from the photographic stimuli to the real people (Stone, 2010). Previous experience of people (i.e. whether positive or negative) creates lasting memories that inform their future interactions with humans (Fureix et al., 2009, Sankey et al., 2010b, Sankey et al., 2010a), and horses demonstrate knowledge of the dominance of familiar conspecifics

and how this relates to their own dominance status. For example, when horses have the opportunity to choose between two regular feeding sites, they avoid the spot of another conspecific (regardless of dominance) if the animal is still present and feeding. However, if the animal is still present but restrained near the food, horses would still avoid the bucket previously chosen by a dominant horse, but return to their own feeding preferences in the presence of a subordinate horse (Krueger and Flauger, 2008).

Horses exhibit sophisticated social skills that are considered complex social behaviour and characteristic of advanced social cognition (Emery, 2005). This refers to skills and behaviours that are seemingly difficult to learn by simple associative processes due to the complexity of the situation, as defined by the high number of variables involved and/or a large range of potential outcomes for each variable. This produces an extensive range of possible combinations for a given circumstance, which would make the chance and likelihood of learning by associative processes unlikely. This is thought to be particularly prevalent in the social environment where individuals represent unpredictable objects, unlike the physical environment that follows predictable laws (Humphrey, 1976, Jolly, 1966). Examples of such skills and behaviours might include coalition and alliance formation, reciprocity and interchange, understanding third party relationships, and reconciliation. It is also suggested to include behaviours that do not have an immediate outcome, for example co-operating, where individuals may have to relinquish short-term gain for long term benefit (Emery, 2005). Correspondingly, after agonistic encounters horses display post-conflict reconciliation, and there is some evidence of third party consolation (Cozzi et al., 2010). Horses may also have some awareness of the relationships between other individuals, as third parties (particularly high ranking mares) may intervene in affiliative interactions between a preferred social partner and other members of the group (Schneider and Krueger, 2012). Interestingly, living in a fission-fusion society where neighbouring groups share space and resources has been suggested to represent some form of cooperation (Aureli et al., 2008), although direct tests of co-operation in horses have not been reported.

Learning and memory

Horses have good abilities for basic discrimination, memory and learning. In experimental tasks horses demonstrate complex pattern discrimination and can learn

relational discriminations using two and three-dimensional objects with open or closed centres, and including relative size among objects (Flannery, 1997, Hanggi, 1999, Hanggi, 2003, Sappington and Goldman, 1994). Therefore horses are able to solve higher order problems using concept formation and generalise this learning under different conditions, which is interesting given their limited and simple physical environment. However, the extent to which horses demonstrate flexibility in learnt responses is unclear. One study has suggested good abilities to reverse learnt responses, with horses learning to discriminate between two boxes containing feed, and then successfully learning daily reversals as to which box contained the feed (Warren and Warren, 1962). However in other cases horses seem to find it difficult to inhibit earlier learnt responses; horses show perseveration in tasks of spatial cognition, sticking with an initially learnt route even when a shorter route becomes available (Osthaus et al., 2013).

Horses do have an excellent long term memory (more than 10 years) and this applies to abstract category and concept formation (Hanggi and Ingersoll, 2009). Strangely, horses' short term memory seems poor and in experimental trials where horses viewed one of two locations being baited with food before being released to make a feeding choice, the introduction of a short, ten second delay into drastically reduced performance compared to immediate release (McLean, 2004). Horses are capable of social learning, and social relationships influence their likelihood of copying another - horses are more likely to copy known dominant individuals than subordinate or unfamiliar horses (Krueger and Heinze, 2008). This social learning can encourage horses to approach an object that they would not otherwise approach, as they are usually highly neophobic of novel objects (Leiner and Fendt, 2011). Other social factors impact the ability of horses to learn, and socially reared and kept horses learn training tasks quicker than those housed individually (Rivera et al., 2002, Søndergaard and Ladewig, 2004).

The perceptual world of the horse

Olfaction

The nostrils of the horse are separated so their openings face different directions, permitting stereo-olfaction for the localisation of olfactory sources (Stoddart, 1980). Horses also use the vomeronasal organ to gain olfactory information, particularly

around the physiological and sexual status of other individuals, in the characteristic upper lip curling known as flehmen (Døving and Trotier, 1998). There appears to be no literature at all on the ability for horses to learn scent discriminations (Saslow, 2002) and it is difficult to evaluate the strength of olfactory perception in the horse due to the lack of research in this area. However, it does seem that olfaction is not the most prominent perceptual cue for horses, as horses react more to an unknown visual and auditory stimulus than to an unknown odour, and they react more to a known odour when combined with an auditory stimulus (Christensen and Rundgren, 2008).

Hearing

Horses hearing ranges between 55Hz and 33,500Hz (compared to a human 31Hz-17,600Hz). Their best sensitivity seems to be around 1000-16,000Hz (peak sensitivity: 2000Hz), although auditory discrimination tasks have not been developed to the same extent as in the visual modality (Heffner, 1998, Heffner and Heffner, 1983). Horses have large mobile ears, which they can move independently and with great specificity. At frequencies below 2000Hz horses use the interaural time difference (the time between the sound reaching the two ears) to locate sound, although it is unclear whether they locate sound at higher frequencies (Heffner and Heffner, 1984, Saslow, 2002). Horses' sound localization thresholds with click stimulus were markedly poorer (30^{o}) than those of other large mammals, such as humans (0.8^{o}) and elephants (1.2^{o}).

Vision

Horses have laterally placed eyes with a nearly 360^{o} field of vision and almost complete decussation of the optic nerves (80-90%). This means that horses have large monocular visual fields and smaller, but not insubstantial ($60\text{-}80^{o}$), binocular vision and stereoscopic abilities (Brooks et al., 1999, Hanggi and Ingersoll, 2012, Harman et al., 1999, Timney and Keil, 1999). It is not conclusively known how horse's vision varies between the binocular and monocular fields of vision (Saslow, 2002). The horse visual system is specialised for detecting contrast and movement, particularly in low light conditions; for example, a 2.54 cm wide stimulus seen on a overcast day was found to be more visible than a 5.08 cm stripe encountered on a sunny day (Prince et al., 1960, Saslow, 2002, Saslow, 1999). Acuity levels for the horse are low compared to humans (horse: 6/19 Snellen notation or 23 cycles per degree, human: 20/20 or 50 cycles per

degree) (Harman et al., 1999, Land and Nilsson, 2012, Timney and Keil, 1992), however this may be an underestimate and horses have better visual acuity than the domestic dog and the domestic cat (Harman et al., 1999, Miller and Murphy, 1995). Additionally, it has been suggested that horses have the physiological mechanisms necessary for accommodation (adjusting the focus of their lens) which dogs do not (Farrall and Handscombe, 1999, Harman et al., 1999).

Despite this ability for accommodation, horses also adjust the height and position of their head to gain optimal use of visual resources (Harman et al., 1999, Saslow, 1999). Horses have an area of binocular overlap directly down the nose (rather than straight ahead), and consequently orient their head towards an object of visual investigation, whilst adjusting the height and angle of the head to the neck for maximum binocular acuity (raising their head high for distant objects, and arching their head or turning their head to one side for closer objects) (Harman et al., 1999). Horses may also move their head in a rhythmic up and down motion (bobbing) to identify stationary visual targets (Saslow, 2002). There is some evidence for a lateral, monocular preference in some emotive situations (De Boyer Des Roches et al., 2008, Farmer et al., 2010, Larose et al., 2006).

Physiological and behavioural data has suggested that horses are blue/yellow dichromats and their sensitivity for cone vision is about the same as humans (Blackmore et al., 2008, Carroll et al., 2001, Macuda and Timney, 1999, Roth et al., 2008). Horses have a horizontal band with a greater density of retinal ganglion cells just below the centre of the eye. Areas of peak density are found within this band immediately above the optic nerve head (Harman et al., 1999, Hebel, 1976). This is an area of specialisation for higher visual acuity presumably for advanced predator detection on the horizon (Hebel, 1976).

Communication

Olfaction

Literature on olfactory communication in horses is sparse. Horses clearly gain some information from chemical cues, taking great interest in dung piles and often investigating unknown individuals and other novel objects using smell (Waring, 2003). Olfactory signals seem to carry some information about identity in horses (although

there are conflicting results about the specific identify information encoded) and may carry information about physiology and sexual status (Krueger and Flauger, 2011, Rubenstein and Hack, 1992, Wolski et al., 1980). However, visual cues seem to be the primary factor attracting stallions to oestrus mares; stallions produced less flehmen when visual access to mares was blocked even though olfactory cues were still available, and blocking vision significantly reduced all aspects of stallions' sexual behaviour to mares compared to when olfaction was blocked (Anderson et al., 1996b).

Vocalisations

The main voiced calls given by horses are the whinny, the squeal, the nicker, and the groan. Horses also produce various non-vocalised sounds such as snorts, blows, and snores. Below I outline the properties and acoustic structure of the three main calls: the whinny, the nicker, and the squeal. Previously these have been identified and discussed as discrete calls. There may be an element of gradation in the vocalisations (pers. obs.), however as this has not been previously reported in the literature and there are no systematic studies to inform the position, only the clearly identifiable, discrete calls will be discussed.

Whinny (neigh)

Whinnies are high amplitude vocalisations that vary in length between 0.5 and 2.8 seconds. The call is considered to have three parts: a squeal-like introduction that is tonal and high frequency; a climax, which is long with frequency and amplitude modulation; and the conclusion, which is low in frequency and amplitude, and pulsates in a manner similar to nickers (Browning and Scheifele, 2005). It was originally reported that the fundamental frequency of whinnies varies between 400 and 2,000 Hz, and may oscillate and gradually decrease throughout the call to around half of the original level (Lemasson et al., 2009, Kiley, 1972). However, recent analysis has suggested that there are two distinct fundamental frequencies, indicative of biphonation. The lower fundamental frequency is around 400Hz (range, 52-1050) and the higher fundamental frequency around 1,500 Hz (range, 493-3012) (Briefer et al., 2015).

Whinnies contain reliable acoustic differences relating to the sex, dominance status, size, and individual identity of horses (but not the age or group affiliation) and in playbacks horses are able to discriminate between individuals on the basis of their whinnies (Lemasson et al., 2009, Proops et al., 2009). Different individuals may exhibit reliable differences in the temporal pattern of whinnies, for example some horses will reliably omit the first or third phases (Lemasson et al., 2009). Whinnies are generally identified as the contact call of horses, and interestingly the different equids have very different contact calls. In the mountain zebra (*E. zebra*) it is a long squeal repeated several times, in plains zebra (*E. quagga*) it is one three-syllable barking sound, and in Grevy's zebra it is an intermittent roar (Klingel, 1977).

Whinnies are responded to when the caller is out of sight and over a quarter of a mile away, and if given by a lost horse whinnies will generally only elicit a response from members of their own group (Feist and McCullough, 1976, Tyler, 1972). Whinnies therefore do seem to have some role in maintaining contact, and are produced in both negative and positive situations. The whinny is often given in greeting after separation from affiliates or offspring, but is also heard as a distress call when horses are separated from close associates (Feist and McCullough, 1976, Tyler, 1972, Waring, 2003). There is a growing body of evidence demonstrating that the acoustic structure of the whinny alters depending on stress levels, however playback recordings to test whether these changes encode meaningful information have not yet been conducted (Briefer et al., 2015, Pond et al., 2010).

Nickers

Nickers are low-pitched broad-band vocalisations with an audible pulsated quality, generally between 0.2 and 1.7s long (Yeon, 2012, Waring, 2003). They are given in situations that seem to represent positive anticipation: prior to feeding; when a stallion is sexually interested in a mare; or between close associates, particularly a mare and her foal. The nicker can be given as a form of greeting to a handler, particularly when food is anticipated, and may also be given to an affiliate conspecific (Houpt, 2005, Kiley, 1972, Waring, 2003). There are some differences between the calls given in the different contexts, the most obvious being that nickers given by mares to their foals are quiet and barely audible beyond the immediate vicinity, whereas nickers given in sexual

anticipation or anticipation of food are loud and often audible at 30m. Although it has been suggested that there are other differences between the nickers given in different contexts, there are no quantitative reports of differences, or playback studies to examine whether these differences are meaningful. Because the call tends to show low tonality, the fundamental frequency is not very apparent and is usually around 100 Hz, and the predominant sound energy is below 200Hz (Kiley, 1972, Waring, 2003, Yeon, 2012).

Squeals

Squeals are high pitched vocalisations that generally last between 0.1 and 1.7s (Yeon, 2012). The fundamental frequency is usually close to 150-200Hz and although higher frequencies are present, most of the sound energy occurs below 4000Hz. Squeals are given in both agonistic and sexual situations (Waring, 2003), although they may also be produced when two horses are introduced for the first time even in the absence of obvious agonistic behaviours (McDonnell and Haviland, 1995)(pers. obs.). Squeals are also occasionally emitted in response to acute pain, such as when an early lactating mare resists being touched on a sore mammary gland or when an injection is being administered (Mills and Nankervis, 2013, Waring, 2003). Nobody has yet investigated whether the squeals given in these contexts vary acoustically.

The squeals of stallions can provide information about dominance status, regardless of familiarity of the call provider and this is recognised by horses in playback experiments (Rubenstein and Hack, 1992). Squeals of dominant horses (those in the top half of the hierarchy) are longer than those of subordinates (those in the bottom half of the hierarchy), and squeals of dominant horses have high frequency components at the call onset that are not present in the squeals of subordinates. Moreover, the squeals of dominant horses maintain three broad energy bands to the end of the call, which is not seen in subordinate horses. This pattern implies that dominant horses are able to squeal for longer and more forcefully than subordinate horses, perhaps due to higher lung capacity. Similarly, the squeals of mares are quantitatively different for mares in oestrus and dioestrus, with oestrus mares typically producing shorter squeals and containing a higher third formant (Kim et al., 2010). Therefore squeals also have the potential to provide information about reproductive status, but no playbacks have yet been conducted to examine whether this is so.

Vision

Overall stance and carriage is believed to indicate mood and physiological condition in horses (Waring, 2003). If attending to something, horses commonly orient their body towards the object of attention, however if a horse is intending or threatening to kick they will bascule their body and orient it posteriorly to the object of attention (McDonnell and Haviland, 1995, Waring, 2003). In relaxation the horses show lowered head, relaxed muscles, and ears relaxed down and laterally, whereas an alert body posture is characterised by a raised head, tensed muscles, and high pricked ears (Waring, 2003). Horse body posture and movement alters when in pain (although specific details are not given), and these changes are used in the veterinary assessment of horses (Bussieres et al., 2008). However whether horses are sensitive to these cues in conspecifics is unknown.

The height of the head and neck may reflect level of attention and/or affect in horses; the head and neck become raised when attending to something, and lowered in relaxation and sleep (Christensen and Rundgren, 2008, Harman et al., 1999, McDonnell and Haviland, 1995, Waring, 2003). It has been noted that a high head and neck position is adopted when attending to far away stimuli (Waring, 2003) and thus the height of the head and neck may also convey information about the proximity of the object of attention. The shape of the neck and angle of the head to the neck is also altered, for example, the head and neck is extended and stretched for tactile and olfactory exploration of objects, held short and high for visual investigation of a distant stimulus, or arched when a horse is focussing on a close object (Harman et al., 1999). An arched neck is generally indicative of negative emotional situations (or potentially high arousal situations), such as when alarmed, meeting a new horse for the first time, or performing stallion rituals (Christensen and Rundgren, 2008, McDonnell and Haviland, 1995, Waring, 2003). It has been suggested that alterations enable optimal use of the sensory receptors and these parameters also influence the balance and locomotion of the horse (Álvarez et al., 2006, Rhodin et al., 2009, Waring, 2003, Weishaupt et al., 2006), so it could be possible that the horse alters the head and neck in preparation for locomotion in an approach or avoidance response to the stimulus.

The ears of the horse are generally considered one of the most important cues to attention and emotional state in horses. Interestingly, ear perking in mammals is suggested to be an evolutionary ancestor of brow movements in humans, which can be conversational signals of emphasis and attention (Ekman and Oster, 1979, Rinn, 1984). A horse's ears generally rotate towards the object of attention (McDonnell and Haviland, 1995, Ransom and Cade, 2009, Waring, 2003). If horses are only mildly attending to a stimulus they may only orient an ear in the direction of attention, however when they are strongly attending to something they will orient both ears forward (McDonnell and Haviland, 1995, Ransom and Cade, 2009, Waring, 2003). The angle of the ears relative to the head may also indicate the level of attention and emotion - when a horse is attending strongly to a stimulus both ears are held taught and upright yet in lower levels of arousal the ears are more relaxed and held obliquely to the head. The ears are one of the final body parts to cease activity and relax when moving from a wakeful state to sleep (Waring, 2003).

When angry, horses flatten their ears to the head and when in pain horses seem to hold their ears in a low and asymmetrical position (Gleerup et al., 2015, Waring, 2003). These emotional indicators alter the general patterns of attentional ear position. When the ears are pinned against the head and neck in agonistic situations, the ears are oriented posteriorly whilst the horse's attention may be oriented anteriorly (Cozzi et al., 2010, McDonnell and Haviland, 1995, Waring, 2003). In another example, retreat situations are generally associated with the ears turned backwards (McDonnell and Haviland, 1995, Waring, 2003). In this situation it is unclear from the observational literature alone whether the ears are orienting towards the object of attention, indicating negative valence, or a combination of both.

Whilst the eyes in horses are considered to hold some communicative information, in the literature this is limited to eyes bright and glossy when healthy and alert, dull and lifeless when unhealthy, partially closed when drowsy or relaxed, and wide open when fearful (Ransom and Cade, 2009, Waring, 2003). Furthermore, the nature of horse vision (e.g. laterally placed eyes and movement of the head for binocular focus) has led people to believe that gross cues are more salient than finer cues, such as eye gaze. Although horses generally have no visible sclera, they do show their sclera in emotive situations (Waring, 2003).

The dilation of the nostrils may also be indicative of attention or arousal, with the nostrils becoming more dilated and 'flaring' as arousal/attention increases (McDonnell and Haviland, 1995, Waring, 2003). This may be a physiological response, to maximise air intake for potential flight responses or olfactory investigation (Franklin et al., 2004, Holcombe et al., 2002, Waring, 2003), however whether other horses are sensitive to this cue has not been established. Position and tension of the lips is associated with attention, affect, and arousal in horses. The mouth is closed and the lips are tense when attending to something (except for in flehmen) however, the mouth is open and the lips (particularly the bottom lip) are relaxed when inattentive and drowsy (Waring, 2003).

Facial Expression

A range of categorised facial expressions in horses has been described by Klingel (1977). This included the 'oestrous face' and 'snapping,' which involve exaggerated chewing movements, retracted lip corners and bared teeth, and ears oriented backwards (estrous face) or sideways (snapping), although these expressions seemed mostly to be separated by whether they were given by an oestrous mare or a young horse. Snapping has also been described by a number of other sources e.g. (McDonnell, 2003, Waring, 2003, pers. obs., Figure 1.) and this may be analogous or homologous to the primate bared teeth gesture. The 'greeting face' involves an extension of the head and neck towards another horse and the ears being directed forwards, while the 'threat expression' varies from a mild threat where the ears are slightly laid back to a serious threat where the ears are flat back, the head is lowered and swaying and the mouth is open. 'Flehmen' is shown in horses, as in many animals, and involves the horse lifting its head up, curling the upper lip to expose the teeth, and lowering the lower lip but keeping the jaw closed. Strangely, despite an ethogram specifically focussing on equid play, and a play face being described in plains zebra, a play face has not been described for horses (McDonnell and Poulin, 2002, Schilder et al., 1984). However, there is a distinctive facial expression given in play (pers. obs., Figure 2).

Unfortunately, since these early descriptions, facial expressions of horses have generally been overlooked or noted in vague terms. For example, although it is frequently suggested that changes in the muscle tension in the face, the position of the

eyes, lips, and contour changes of the skin on the face are important in equine communication (Waring, 2003) these have not been systematically documented. McDonnell & Haviland (1995) admit that this is a weakness of their previous research and highlight the need for further work to be done in this area. Interestingly, a quantitative analysis of facial expressions has been attempted for plains zebra, which has demonstrated a number of distinct facial expressions given in different contexts (Schilder et al., 1984). It is surprising that this has not been achieved with horses, given their domestic and popular status.

Section summary

Horses live under the same social demands that are thought to have driven the evolution of advanced cognition and communication in other species. Correspondingly, they exhibit some of the socio-cognitive skills considered necessary for dealing with social challenges. Horses can perform individual recognition of social partners; they have good long-term memories; they demonstrate knowledge of their relationships with others, and seek to maintain valuable social relationships through mechanisms such as post conflict reconciliation; they also appear to recognise relationships between third parties. However, their ability to gain information from social partners is less well understood, particularly in the visual modality – their strongest perceptual sense. Previous work has demonstrated that horses can gain some information from human social partners, however information transfer in conspecific systems has been largely overlooked. In this thesis I address this by examining the production and perception of a key method of visual communication: facial expressions.

Horses are an ideal model species to allow us to evaluate the contribution of selective pressures to the development of their cognition and communication. As they have a simple foraging environment it is unlikely that their advanced cognitive abilities evolved to help them meet these demands, but more likely that they evolved to assist individuals in navigating their complex social world. Horses also make an excellent comparative model, as they are very distantly related to primates, which are one of the most studied taxa. Therefore, similarities seen between these diverse taxonomic groups are likely to be the result of convergent evolution under common selective pressures, or else they represent evolutionarily old and highly conserved systems.

Aims and Research Questions

The general aim of this thesis is to contribute to knowledge of social cognition and communication by conducting a body of research examining the production and perception of facial expressions in the domestic horse. Specifically, this work aims to: explore the evolution of communicative systems through the visual modality of facial expressions; investigate social understanding and how this may help animals navigate their environment; and through this, gain insights into the cognitive and neurological mechanisms that aid communication and social cognition.

In the following empirical chapters, I address the specific questions below that relate to these broad areas of social cognition and communication:

1. Do horses have the capacity to produce complex facial expressions?
2. Can the production of facial expressions in horses offer insights into their cognitive and neurological representation of social information?
3. Are horses sensitive to the attentional state of a conspecific and does this inform their foraging decisions?
4. Are horses sensitive to the emotional state of a conspecific and does this inform their social decisions?

References

Adolphs, R. 2010. Emotion. *Current Biology,* 20**,** R549-R552.

Aiello, L. C. & Wheeler, P. 1995. The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. *Current Anthropology,* 36**,** 199-221.

Álvarez, C. B. G., Rhodin, M., Bobbert, M. F., Meyer, H., Weishaupt, M. A., Johnston, C. & Van Weeren, P. R. 2006. The effect of head and neck position on the thoracolumbar kinematics in the unridden horse. *Equine Veterinary Journal,* 38**,** 445-451.

Anderson, J. R., Montant, M. & Schmitt, D. 1996a. Rhesus monkeys fail to use gaze direction as an experimenter-given cue in an object-choice task. *Behavioural processes,* 37**,** 47-55.

Anderson, J. R., Sallaberry, P. & Barbier, H. 1995. Use of experimenter-given cues during object-choice tasks by capuchin monkeys. *Animal Behaviour,* 49**,** 201-208.

Anderson, T. M., Pickett, B. W., Heird, J. C. & Squires, E. L. 1996b. Effect of blocking vision and olfaction on sexual responses of haltered or loose stallions. *Journal of Equine Veterinary Science,* 16**,** 254-261.

Andrew, R. J. 1963a. Evolution of Facial Expression. *Science,* 142**,** 1034-1041.

Andrew, R. J. 1963b. The origin and evolution of the calls and facial expressions of the primates. *Behaviour,* 20**,** 1-107.

Armstrong, E. 1982. A look at relative brain size in mammals. *Neuroscience Letters,* 34**,** 101-104.

Armstrong, E. 1983. Relative brain size and metabolism in mammals. *Science,* 220**,** 1302-1304.

Aschersleben, G., Hofer, T. & Jovanovic, B. 2008. The link between infant attention to goal-directed action and later theory of mind abilities. *Developmental science,* 11**,** 862-8.

Aureli, F., Schaffner, C. M., Boesch, C., Bearder, S. K., Call, J., Chapman, C. A., Connor, R., Fiore, A. D., Dunbar, R. I. M., Henzi, S. P., Holekamp, K., Korstjens, A. H., Layton, R., Lee, P., Lehmann, J., Manson, J. H., Ramos-Fernandez, G., Strier, K. B. & Van Schaik, C. P. 2008. Fission-Fusion Dynamics: New Research Frameworks. *Current Anthropology,* 49**,** 627-654.

Aviezer, H., Trope, Y. & Todorov, A. 2012. Body cues, not facial expressions, discriminate between intense positive and negative emotions. *Science,* 338**,** 1225-9.

Baldock, N. M. & Sibly, R. M. 1990. Effects of handling and transportation on the heart rate and behaviour of sheep. *Applied Animal Behaviour Science,* 28**,** 15-39.

Barrett, L. F. 2006. Are emotions natural kinds? *Perspectives on Psychological Science,* 1**,** 28-58.

Barrett, L. F., Lindquist, K. A., Bliss-Moreau, E., Duncan, S., Gendron, M., Mize, J. & Brennan, L. 2007. Of mice and men: Natural kinds of emotions in the mammalian brain? A response to Panksepp and Izard. *Perspectives on Psychological Science,* 2**,** 297-312.

Bartal, I. B.-A., Decety, J. & Mason, P. 2011. Empathy and Pro-Social Behavior in Rats. *Science,* 334**,** 1427-1430.

Barth, J., Reaux, J. & Povinelli, D. 2005. Chimpanzees (Pan troglodytes) use of gaze cues in object-choice tasks: different methods yield different results. *Animal cognition,* 8**,** 84-92.

Barton, R. A. 2006. Primate brain evolution: Integrating comparative, neurophysiological, and ethological data. *Evolutionary Anthropology: Issues, News, and Reviews,* 15**,** 224-236.

Bearder, S. K., Nekaris, K. a. I. & Curtis, D. J. 2006. A Re-Evaluation of the Role of Vision in the Activity and Communication of Nocturnal Primates. *Folia Primatologica,* 77**,** 50-71.

Berger, J. 1986. *Wild Horses of the Great Basin: Social Competition and Population Size,* University of Chicago Press.

Bindemann, M., Mike Burton, A. & Langton, S. R. H. 2008. How do eye gaze and facial expression interact? *Visual Cognition,* 16**,** 708-733.

Blackmore, T. L., Foster, T. M., Sumpter, C. E. & Temple, W. 2008. An investigation of colour discrimination with horses (Equus caballus). *Behavioural processes,* 78**,** 387-96.

Boissy, A., Aubert, A., Desire, L., Greiveldinger, L., Delval, E. & Veissier, I. 2011. Cognitive sciences to relate ear postures to emotions in sheep. *Animal Welfare,* 20**,** 47.

Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I. & Aubert, A. 2007. Assessment of positive emotions in animals to improve their welfare. *Physiology & Behavior,* 92**,** 375-97.

Bolwig, N. 1964. Facial Expression in Primates With Remarks On a Parallel Development in Certain Carnivores (a Preliminary Report On Work in Progress). *Behaviour,* 22**,** 167-192.

Bourjade, M., Meguerditchian, A., Maille, A., Gaunet, F. & Vauclair, J. 2014. Olive baboons, Papio anubis, adjust their visual and auditory intentional gestures to the visual attention of others. *Animal Behaviour,* 87**,** 121-128.

Bräuer, J., Call, J. & Tomasello, M. 2004. Visual perspective taking in dogs (Canis familiaris) in the presence of barriers. *Applied Animal Behaviour Science,* 88**,** 299-317.

Bräuer, J., Call, J. & Tomasello, M. 2005. All great ape species follow gaze to distant locations and around barriers. *Journal of Comparative Psychology,* 119**,** 145.

Bray, J., Krupenye, C. & Hare, B. 2014. Ring-tailed lemurs (Lemur catta) exploit information about what others can see but not what they can hear. *Animal cognition,* 17**,** 735-744.

Briefer, E. F. 2012. Vocal expression of emotions in mammals: mechanisms of production and evidence. *Journal of Zoology,* 288**,** 1-20.

Briefer, E. F., Maigrot, A. L., Mandel, R., Freymond, S. B., Bachmann, I. & Hillmann, E. 2015. Segregation of information about emotional arousal and valence in horse whinnies. *Scientific reports,* 4**,** 9989.

Brooks, Komàromy & Källberg 1999. Comparative retinal ganglion cell and optic nerve morphology. *Veterinary Ophthalmology,* 2**,** 3-11.

Brown, C. R. 1988. Enhanced foraging efficiency through information centers: a benefit of coloniality in cliff swallows. *Ecology***,** 602-613.

Browning, D. G. & Scheifele, P. M. 2005. Equine acoustics: Anatomy of a whinny. *The Journal of the Acoustical Society of America,* 118**,** 2001-2001.

Bruce, V. & Young, A. 2013. *Face Perception,* Taylor & Francis.

Buchanan, T. W., Al'absi, M. & Lovallo, W. R. 1999. Cortisol fluctuates with increases and decreases in negative affect. *Psychoneuroendocrinology,* 24**,** 227-241.

Bugnyar, T. & Kotrschal, K. 2002. Observational learning and the raiding of food caches in ravens, Corvus corax: is it 'tactical' deception? *Animal Behaviour,* 64**,** 185-195.

Bugnyar, T. & Kotrschal, K. 2004. Leading a conspecific away from food in ravens (Corvus corax)? *Animal cognition,* 7**,** 69-76.

Bulloch, M. J., Boysen, S. T. & Furlong, E. E. 2008. Visual attention and its relation to knowledge states in chimpanzees, Pan troglodytes. *Animal Behaviour,* 76**,** 1147-1155.

Burgdorf, J. & Panksepp, J. 2006. The neurobiology of positive emotions. *Neuroscience and biobehavioral reviews,* 30**,** 173-87.

Burger, J., Gochfeld, M. & Murray, B. G. 1992. Risk discrimination of eye contact and directness of approach in black iguanas (Ctenosaura similis). *Journal of Comparative Psychology,* 106**,** 97.

Burghardt, G. M. & Greene, H. W. 1988. Predator simulation and duration of death feigning in neonate hognose snakes. *Animal Behaviour,* 36**,** 1842-1844.

Burrows, A. M. 2008. The facial expression musculature in primates and its evolutionary significance. *BioEssays,* 30**,** 212-225.

Burrows, A. M., Diogo, R., Waller, B. M., Bonar, C. J. & Liebal, K. 2011. Evolution of the Muscles of Facial Expression in a Monogamous Ape: Evaluating the Relative Influences of Ecological and Phylogenetic Factors in Hylobatids. *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology,* 294**,** 645-663.

Burrows, A. M. & Smith, T. D. 2003. Muscles of facial expression in Otolemur, with a comparison to lemuroidea. *The anatomical record. Part A, Discoveries in molecular, cellular, and evolutionary biology,* 274**,** 827-36.

Burrows, A. M., Waller, B. M. & Parr, L. A. 2009. Facial musculature in the rhesus macaque (Macaca mulatta): evolutionary and functional contexts with comparisons to chimpanzees and humans. *Journal of Anatomy,* 215**,** 320-334.

Burrows, A. M., Waller, B. M., Parr, L. A. & Bonar, C. J. 2006. Muscles of facial expression in the chimpanzee (Pan troglodytes): descriptive, comparative and phylogenetic contexts. *Journal of Anatomy,* 208**,** 153-167.

Bussieres, G., Jacques, C., Lainay, O., Beauchamp, G., Leblond, A., Cadore, J. L., Desmaizieres, L. M., Cuvelliez, S. G. & Troncy, E. 2008. Development of a composite orthopaedic pain scale in horses. *Research in Veterinary Science,* 85**,** 294-306.

Byrne, R. W. 1995. *The Thinking Ape: Evolutionary Origins of Intelligence*, Oxford University Press.

Byrne, R. W. & Bates, L. A. 2007. Sociality, evolution and cognition. *Current biology : CB,* 17**,** R714-23.

Byrne, R. W. & Whiten, A. 1988. *Machiavellian Intelligence: Social Expertise and the Evolution of Intellect in Monkeys, Apes, and Humans*, Clarendon Press.

Cacioppo, J. T. & Gardner, W. L. 1999. Emotion. *Annual Review of Psychology,* 50**,** 191-214.

Caeiro, C. C., Burrows, A.M., Waller, B.M. 2013. CatFACS. Univeristy of Portsmouth.

Call, J., Bräuer, J., Kaminski, J. & Tomasello, M. 2003. Domestic dogs (Canis familiaris) are sensitive to the attentional state of humans. *Journal of Comparative Psychology,* 117**,** 257.

Call, J., Hare, B. A. & Tomasello, M. 1998. Chimpanzee gaze following in an object-choice task. *Animal cognition,* 1**,** 89-99.

Call, J. & Tomasello, M. 1998. Distinguishing intentional from accidental actions in orangutans (Pongo pygmaeus), chimpanzees (Pan troglodytes) and human children (Homo sapiens). *Journal of Comparative Psychology,* 112**,** 192.

Cameron, E. Z., Setsaas, T. H. & Linklater, W. L. 2009. Social bonds between unrelated females increase reproductive success in feral horses. *Proceedings of the National Academy of Sciences of the United States of America,* 106**,** 13850-3.

Cannon, W. B. 1914. Recent Studies of Bodily Effects of Fear, Rage, and Pain. *The Journal of Philosophy, Psychology and Scientific Methods,* 11**,** 162-165.

Carroll, J., Murphy, C. J., Neitz, M., Hoeve, J. N. & Neitz, J. 2001. Photopigment basis for dichromatic color vision in the horse. *J Vis,* 1**,** 80-7.

Cheney, D. L. & Seyfarth, R. M. 2008. *Baboon Metaphysics: The Evolution of a Social Mind*, University of Chicago Press.

Chevalier-Skolnikoff, S. 1973. Facial expressions of emotion in non-human primates. *In:* Ekman, P. (ed.) *Darwin and Facial Expressions.* New York: Acadamy Press.

Christensen, J. W. & Rundgren, M. 2008. Predator odour per se does not frighten domestic horses. *Applied Animal Behaviour Science,* 112**,** 136-145.

Christensen, J. W., Zharkikh, T., Ladewig, J. & Yasinetskaya, N. 2002. Social behaviour in stallion groups (Equus przewalskii and Equus caballus) kept under natural and domestic conditions. *Applied Animal Behaviour Science,* 76**,** 11-20.

Chun, M. M. 2000. Contextual cueing of visual attention. *Trends in cognitive sciences,* 4**,** 170-178.

Clutton-Brock, J. 1999. *A Natural History of Domesticated Mammals*, Cambridge University Press.

Clutton-Brock, T. H., Brotherton, P. N. M., O'riain, M. J., Griffin, A. S., Gaynor, D., Kansky, R., Sharpe, L. & Mcilrath, G. M. 2001. Contributions to cooperative rearing in meerkats. *Animal Behaviour,* 61**,** 705-710.

Clutton-Brock, T. H. & Harvey, P. H. 1980. Primates, brains and ecology. *Journal of Zoology,* 190**,** 309-323.

Connor, R. C., Mann, J., Tyack, P. L. & Whitehead, H. 1998. Social evolution in toothed whales. *Trends in ecology & evolution,* 13**,** 228-232.

Council of the European Union. 1997. Treaty on European Union (Consolidated Version), Treaty of Amsterdam.

Cozzi, A., Sighieri, C., Gazzano, A., Nicol, C. J. & Baragli, P. 2010. Post-conflict friendly reunion in a permanent group of horses (Equus caballus). *Behavioural processes,* 85**,** 185-90.

Crowell-Davis, S. L. 1993. Social behaviour of the horse and its consequences for domestic management. *Equine Veterinary Education,* 5**,** 148-150.

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E. & Leach, M. C. 2014. Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration. *PLoS one,* 9**,** e92281.

Dally, J. M., Emery, N. J. & Clayton, N. S. 2005. Cache protection strategies by western scrub-jays, Aphelocoma californica: implications for social cognition. *Animal Behaviour,* 70**,** 1251-1263.

Danchin, E., Giraldeau, L. A., Valone, T. J. & Wagner, R. H. 2004. Public information: from nosy neighbors to cultural evolution. *Science,* 305**,** 487-91.

Darwin, C. 1999. *The Expression of the Emotions in Man and Animals,* London, UK, Fontana Press.

Davidson, R. J., Jackson, D. C. & Kalin, N. H. 2000. Emotion, plasticity, context, and regulation: perspectives from affective neuroscience. *Psychological Bulletin,* 126**,** 890.

Davila Ross, M., Owren, M. J. & Zimmermann, E. 2009. Reconstructing the evolution of laughter in great apes and humans. *Current biology : CB,* 19**,** 1106-11.

De Boyer Des Roches, A., Richard-Yris, M. A., Henry, S., Ezzaouia, M. & Hausberger, M. 2008. Laterality and emotions: visual laterality in the domestic horse (Equus caballus) differs with objects' emotional value. *Physiology & Behavior,* 94**,** 487-90.

De Waal, F. B. 1988. The communicative repertoire of captive bonobos (Pan paniscus), compared to that of chimpanzees. *Behaviour,* 106**,** 183-251.

De Waal, F. B. 2011. What is an animal emotion? *Annals of the New York Academy of Sciences,* 1224**,** 191-206.

De Waal, F. B. M. 2003. Darwin's Legacy and the Study of Primate Visual Communication. *Annals of the New York Academy of Sciences,* 1000**,** 7-31.

Defensor, E. B., Corley, M. J., Blanchard, R. J. & Blanchard, D. C. 2012. Facial expressions of mice in aggressive and fearful contexts. *Physiology & Behavior,* 107**,** 680-685.

Dennett, D. 1987. *The intentional stance,* Cambridge, MA, USA., MIT Press.

Désiré, L., Boissy, A. & Veissier, I. 2002. Emotions in farm animals: a new approach to animal welfare in applied ethology. *Behavioural processes,* 60**,** 165-180.

Dimberg, U., Thunberg, M. & Elmehed, K. 2000. Unconscious Facial Reactions to Emotional Facial Expressions. *Psychological science,* 11**,** 86-89.

Diogo, R. & Wood, B. A. 2012. *Comparative Anatomy and Phylogeny of Primate Muscles and Human Evolution.* Science Publishers.

Diogo, R., Wood, B. A., Aziz, M. A. & Burrows, A. 2009. On the origin, homologies and evolution of primate facial muscles, with a particular focus on hominoids and a suggested unifying nomenclature for the facial muscles of the Mammalia. *Journal of Anatomy,* 215**,** 300-19.

Dobson, S. D. 2009a. Allometry of facial mobility in anthropoid primates: implications for the evolution of facial expression. *American journal of physical anthropology,* 138**,** 70-81.

Dobson, S. D. 2009b. Socioecological correlates of facial mobility in nonhuman anthropoids. *American journal of physical anthropology,* 139**,** 413-20.

Dobson, S. D. 2012. Face to face with the social brain. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 367**,** 1901-8.

Døving, K. B. & Trotier, D. 1998. Structure and function of the vomeronasal organ. *The Journal of experimental biology,* 201**,** 2913-2925.

Dunbar, R. I. 2003. The social brain: mind, language, and society in evolutionary perspective. *Annual Review of Anthropology***,** 163-181.

Dunbar, R. I. & Shultz, S. 2007. Evolution in the social brain. *Science,* 317**,** 1344-7.

Dunbar, R. I. M. 1993. Coevolution of neocortical size, group size and language in humans. *Behavioral and Brain Sciences,* 16**,** 681-694.

Dunbar, R. I. M. & Bever, J. 1998. Neocortex Size Predicts Group Size in Carnivores and Some Insectivores. *Ethology,* 104**,** 695-708.

Dunn, P. O., Cockburn, A. & Mulder, R. A. 1995. *Fairy-Wren Helpers often Care for Young to Which they are Unrelated*.

Ehmer, B., Reeve, H. K. & Hoy, R. R. 2001. Comparison of brain volumes between single and multiple foundresses in the paper wasp Polistes dominulus. *Brain, behavior and evolution,* 57**,** 161-168.

Ekman, P. (ed.) 1999. *Facial Expressions,* Chichester, UK: John Wiley & Sons, Inc.

Ekman, P., Davidson, R. J. & Friesen, W. V. 1990. The Duchenne smile: emotional expression and brain physiology. II. *J Pers Soc Psychol,* 58**,** 342-53.

Ekman, P. & Friesen, W. V. 1971. Constants across cultures in the face and emotion. *Journal of personality and social psychology,* 17**,** 124.

Ekman, P., Friesen, W. V. & Hager, J. C. 2002. *Facial Action Coding System,* Salt Lake City, Research Nexus.

Ekman, P., Levenson, R. & Friesen, W. 1983. Autonomic nervous system activity distinguishes among emotions. *Science,* 221**,** 1208-1210.

Ekman, P. & Oster, H. 1979. Facial Expressions of Emotion. *Annual Review of Psychology,* 30**,** 527-554.

Ekman, P., Sorenson, E. R. & Friesen, W. V. 1969. Pan-Cultural Elements in Facial Displays of Emotion. *Science,* 164**,** 86-88.

Elfenbein, H. A. & Ambady, N. 2002. On the universality and cultural specificity of emotion recognition: a meta-analysis. *Psychological Bulletin,* 128**,** 203.

Elfenbein, H. A. & Ambady, N. 2003. Universals and Cultural Differences in Recognizing Emotions. *Current Directions in Psychological Science,* 12**,** 159-164.

Emery, N. J. 2000. The eyes have it: the neuroethology, function and evolution of social gaze. *Neuroscience and Biobehavioral Reviews,* 24**,** 581-604.

Emery, N. J. 2005. The evolution of social cognition. *In:* Easton, A. & Emery, N. J. (eds.) *The Cognitive Neuroscience of Social Behaviour.* Hove, UK: Psychology Press.

Emery, N. J., Dally, J. M. & Clayton, N. S. 2004. Western scrub-jays (Aphelocoma californica) use cognitive strategies to protect their caches from thieving conspecifics. *Animal cognition,* 7**,** 37-43.

Fagot, J. & Deruelle, C. 2002. Perception of pictorial eye gaze by baboons (Papio papio). *Journal of Experimental Psychology: Animal Behavior Processes,* 28**,** 298.

Farmer, K., Krueger, K. & Byrne, R. W. 2010. Visual laterality in the domestic horse (Equus caballus) interacting with humans. *Animal cognition,* 13**,** 229-38.

Farrall, H. & Handscombe, M. 1999. Equine vision. *Equine Veterinary Journal,* 31**,** 354-355.

Feh, C. & De Maziéres, J. 1993. Grooming at a preferred site reduces heart rate in horses. *Animal Behaviour,* 46**,** 1191-1194.

Feist, J. D. & Mccullough, D. R. 1976. Behavior Patterns and Communication in Feral Horses. *Zeitschrift für Tierpsychologie,* 41**,** 337-371.

Flack, J. C. & De Waal, F. 2007. Context modulates signal meaning in primate communication. *Proceedings of the National Academy of Sciences of the United States of America,* 104**,** 1581-6.

Flannery, B. 1997. Relational discrimination learning in horses. *Applied Animal Behaviour Science,* 54**,** 267-280.

Forkman, B., Boissy, A., Meunier-Salaün, M. C., Canali, E. & Jones, R. B. 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior,* 92**,** 340-374.

Fox, M. W. 1970. A Comparative Study of the Development of Facial Expressions in Canids; Wolf, Coyote and Foxes. *Behaviour,* 36**,** 49-73.

Franklin, S. H., Price, C. & Burn, J. F. 2004. The displaced equine soft palate as a source of abnormal respiratory noise during expiration. *Equine Veterinary Journal,* 36**,** 590-594.

Fredrickson, B. L. 2004. The broaden-and-build theory of positive emotions. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 359**,** 1367-78.

Freeberg, T. M., Krama, T., Vrublevska, J., Krams, I. & Kullberg, C. 2014. Tufted titmouse (Baeolophus bicolor) calling and risk-sensitive foraging in the face of threat. *Animal cognition,* 17**,** 1341-1352.

Frijda, N. H. 1986. *The Emotions,* Cambridge University Press.

Frijda, N. H. 2009. Emotion Experience and its Varieties. *Emotion Review,* 1**,** 264-271.

Fureix, C., Jego, P., Sankey, C. & Hausberger, M. 2009. How horses (Equus caballus) see the world: humans as significant "objects". *Animal cognition,* 12**,** 643-54.

Genty, E., Breuer, T., Hobaiter, C. & Byrne, R. W. 2009. Gestural communication of the gorilla (Gorilla gorilla): repertoire, intentionality and possible origins. *Animal cognition,* 12**,** 527-46.

Gleerup, K. B., Forkman, B., Lindegaard, C. & Andersen, P. H. 2015. An equine pain face. *Veterinary anaesthesia and analgesia,* 42**,** 103-14.

Glenk, L. M., Kothgassner, O. D., Stetina, B. U., Palme, R., Kepplinger, B. & Baran, H. 2013. Therapy dogs' salivary cortisol levels vary during animal-assisted interventions. *Animal Welfare,* 22**,** 369-378.

Goodall, J. 1986. *The Chimpanzees of Gombe: Patterns of Behaviour,* Bellknap Press of the Harvard University Press.

Goodwin, D. 2002. Horse behaviour: evolution, domestication, and feralisation. *In:* Waran, N. (ed.) *The Welfare of Horses.* Dordrecht: Kluwer.

Gregory, W. K. 1929. *Our Face from Fish to Man: A Portrait Gallery of Our Ancient Ancestors and Kinsfolk Together with a Concise History of Our Best Features,* G.P. Putnam's Sons.

Hanggi, E. B. 1999. Interocular transfer of learning in horses (Equus caballus). *Journal of Equine Veterinary Science,* 19**,** 518-524.

Hanggi, E. B. 2003. Discrimination learning based on relative size concepts in horses (Equus caballus). *Applied Animal Behaviour Science,* 83**,** 201-213.

Hanggi, E. B. & Ingersoll, J. F. 2009. Long-term memory for categories and concepts in horses (Equus caballus). *Animal cognition,* 12**,** 451-462.

Hanggi, E. B. & Ingersoll, J. F. 2012. Lateral vision in horses: A behavioral investigation. *Behavioural processes,* 91**,** 70-76.

Harding, E. J., Paul, E. S. & Mendl, M. 2004. Cognitive bias and affective state. *Nature,* 427**,** 247.

Hare, B. 2001. Can competitive paradigms increase the validity of experiments on primate social cognition? *Animal cognition,* 4**,** 269-280.

Hare, B., Brown, M., Williamson, C. & Tomasello, M. 2002. The domestication of social cognition in dogs. *Science,* 298**,** 1634-6.

Hare, B., Call, J. & Tomasello, M. 2001. Do chimpanzees know what conspecifics know? *Animal Behaviour,* 61**,** 139-151.

Hare, B., Call, J. & Tomasello, M. 2006. Chimpanzees deceive a human competitor by hiding. *Cognition,* 101**,** 495-514.

Hare, B., Plyusnina, I., Ignacio, N., Schepina, O., Stepika, A., Wrangham, R. & Trut, L. 2005. Social cognitive evolution in captive foxes is a correlated by-product of experimental domestication. *Current biology : CB,* 15**,** 226-30.

Hare, B. & Tomasello, M. 1999. Domestic dogs (Canis familiaris) use human and conspecific social cues to locate hidden food. *Journal of Comparative Psychology,* 113**,** 173.

Hare, B. & Tomasello, M. 2005. Human-like social skills in dogs? *Trends in cognitive sciences,* 9**,** 439-44.

Harman, A. M., Moore, S., Hoskins, R. & Keller, P. 1999. Horse vision and an explanation for the visual behaviour originally explained by the 'ramp retina'. *Equine Veterinary Journal,* 31**,** 384-390.

Harvey, P. H. & Clutton-Brock, T. H. 1985. Life history variation in primates. *Evolution***,** 559-581.

Hebel, R. 1976. Distribution of retinal ganglion cells in five mammalian species (pig, sheep, ox, horse, dog). *Anatomy and Embryology,* 150**,** 45-51.

Hecht, E. E., Patterson, R. & Barbey, A. K. 2012. What can other animals tell us about human social cognition? An evolutionary perspective on reflective and reflexive processing. *Frontiers in human neuroscience,* 6**,** 224.

Heffner, H. E. 1998. Auditory awareness. *Applied Animal Behaviour Science,* 57**,** 259-268.

Heffner, H. E. & Heffner, R. S. 1984. Sound localization in large mammals: Localization of complex sounds by horses. *Behavioral Neuroscience,* 98**,** 541.

Heffner, R. S. & Heffner, H. E. 1983. Hearing in large mammals: Horses (Equus caballus) and cattle (Bos taurus). *Behavioral Neuroscience,* 97**,** 299.

Held, S., Mendl, M., Devereux, C. & Byrne, R. W. 2001. Behaviour of domestic pigs in a visual perspective taking task. *Behaviour,* 138**,** 1337 - 1354.

Hennig, C. W. 1977. Effects of simulated predation on tonic immobility in Anolis carolinensis: The role of eye contact. *Bulletin of the Psychonomic Society,* 9**,** 239-242.

Hobaiter, C. & Byrne, R. 2011. The gestural repertoire of the wild chimpanzee. *Animal cognition,* 14**,** 745-767.

Holcombe, S. J., Berney, C., Cornelisse, C. J., Derksen, F. J. & Robinson, N. E. 2002. Effect of commercially available nasal strips on airway resistance in exercising horses. *American Journal of Veterinary Research,* 63**,** 1101-1105.

Hole, G. & Bourne, V. 2010. *Face Processing: Psychological, Neuropsychological, and Applied Perspectives*, OUP Oxford.

Horowitz, A. 2009. Attention to attention in domestic dog (Canis familiaris) dyadic play. *Animal cognition,* 12**,** 107-118.

Houpt, K. A. 2005. *Domestic Animal Behaviour for Veterinarians and Animal Scientists,* Oxford, UK, Blackwell Publishing.

Huber, E. 1931. *Evolution of facial musculature and expression,* Baltimore, John Hopkins Press.

Humphrey, N. 1976. The social function of intellect. *In:* Bateson, P. P. G. & Hinde, R. A. (eds.) *Growing Points in Ethology.* Cambridge: Cambridge University Press.

Itakura, S. 1996. An exploratory study of gaze-monitoring in nonhuman primates1. *Japanese Psychological Research,* 38**,** 174-180.

Izard, C. E. 1994. Innate and universal facial expressions: evidence from developmental and cross-cultural research. *Pyschological Bulletin,* 115**,** 288-299.

Jochle, W., Moore, J., Brown, J., Baker, G., Lowe, J., Fubini, S., Reeves, M., Watkins, J. & White, N. 1989. Comparison of detomidine, butorphanol, flunixin meglumine and xylazine in clinical cases of equine colic. *Equine Veterinary Journal,* 21**,** 111-116.

Jolly, A. 1966. Lemur social behaviour and primate intelligence. *Science,* 153**,** 510-506.

Jolly, A. 2004. *Lords and Lemurs: Mad Scientists, Kings with Spears, and the Survival of Diversity in Madagascar,* Houghton Mifflin.

Julian, G. E. & Gronenberg, W. 2001. Reduction of brain volume correlates with behavioral changes in queen ants. *Brain, behavior and evolution,* 60**,** 152-164.

Kaminski, J., Call, J. & Tomasello, M. 2004. Body orientation and face orientation: two factors controlling apes' behavior from humans. *Animal cognition,* 7**,** 216-23.

Kaminski, J., Pitsch, A. & Tomasello, M. 2013. Dogs steal in the dark. *Animal cognition,* 16**,** 385-94.

Kaminski, J., Riedel, J., Call, J. & Tomasello, M. 2005. Domestic goats, Capra hircus, follow gaze direction and use social cues in an object choice task. *Animal Behaviour,* 69**,** 11-18.

Kaplan, G. 2011. Pointing gesture in a bird,Äìmerely instrumental or a cognitively complex behavior. *Current Zoology,* 57**,** 453-467.

Kaufman, J. A. 2003. On the Expensive Tissue Hypothesis: Independent Support from Highly Encephalized Fish. *Current Anthropology,* 44**,** 705-707.

Keating, S. C., Thomas, A. A., Flecknell, P. A. & Leach, M. C. 2012. Evaluation of EMLA cream for preventing pain during tattooing of rabbits: changes in physiological, behavioural and facial expression responses. *PLoS one,* 7**,** e44437.

Kiley, M. 1972. The Vocalizations of Ungulates, their Causation and Function. *Zeitschrift für Tierpsychologie,* 31**,** 171-222.

Kiltie, R. 2000. Scaling of visual acuity with body size in mammals and birds. *Functional Ecology,* 14**,** 226-234.

Kim, Y. K., Seo, E. G., Lee, S. S., Suh, E. H., Houpt, K. A., Lee, H. C., Lee, H. J. & Yeon, S. C. 2010. Comparative Analysis of Vocalizations of Thoroughbred Mares (Equus caballus) between Estrus and Diestrus. *Journal of Veterinary Medical Science,* 72**,** 929-933.

Kingdon, J. 2007. Primate Visual Signals in Noisy Environments. *Folia Primatologica,* 78**,** 389-404.

Kipper, S. & Todt, D. 2002. The use of vocal signals in the social play of Barbary macaques. *Primates,* 43**,** 3-17.

Klingel, H. 1974. Social organization and behavior of Grevy's zebra (Equus grevyi). *Z Tierpsychol,* 36**,** 37-70.

Klingel, H. 1975. Social organization and reproduction in equids. *Journal of reproduction and fertility. Supplement***,** 7.

Klingel, H. 1977. Communication in Perissodactyla. *In:* Sebeok, T. A. (ed.) *How Animals Communicate.* Bloomington & London: Indiana University Press.

Knutson, B., Burgdorf, J. & Panksepp, J. 2002. Ultrasonic vocalizations as indices of affective states in rats. *Psychological Bulletin,* 128**,** 961-977.

Kobayashi, H. & Kohshima, S. 1997. Unique morphology of the human eye. *Nature,* 387**,** 767-768.

Kobayashi, H. & Kohshima, S. 2001. Unique morphology of the human eye and its adaptive meaning: comparative studies on external morphology of the primate eye. *Journal of human evolution,* 40**,** 419-435.

Krueger, K. & Flauger, B. 2008. Social feeding decisions in horses (Equus caballus). *Behavioural processes,* 78**,** 76-83.

Krueger, K. & Flauger, B. 2011. Olfactory recognition of individual competitors by means of faeces in horse (Equus caballus). *Animal cognition,* 14**,** 245-57.

Krueger, K., Flauger, B., Farmer, K. & Hemelrijk, C. 2014. Movement initiation in groups of feral horses. *Behavioural processes,* 103**,** 91-101.

Krueger, K., Flauger, B., Farmer, K. & Maros, K. 2011. Horses (Equus caballus) use human local enhancement cues and adjust to human attention. *Animal cognition,* 14**,** 187-201.

Krueger, K. & Heinze, J. 2008. Horse sense: social status of horses (*Equus caballus*) affects their likelihood of copying other horses' behavior. *Animal cognition,* 11**,** 431-439.

Lack, D. 1943. *The Life of the Robin,* London, UK, H.F. & G. Whiterby Limited.

Lampe, J. F. & Andre, J. 2012. Cross-modal recognition of human individuals in domestic horses (Equus caballus). *Animal cognition.*

Land, M. F. & Nilsson, D. E. 2012. *Animal Eyes,* OUP Oxford.

Langford, D. J., Bailey, A. L., Chanda, M. L., Clarke, S. E., Drummond, T. E., Echols, S., Glick, S., Ingrao, J., Klassen-Ross, T., Lacroix-Fralish, M. L., Matsumiya, L., Sorge, R. E., Sotocinal, S. G., Tabaka, J. M., Wong, D., Van Den Maagdenberg, A. M. J. M., Ferrari, M. D., Craig, K. D. & Mogil, J. S. 2010. Coding of facial expressions of pain in the laboratory mouse. *Nat Meth,* 7**,** 447-449.

Langton, S. R. H., Watt, R. J. & Bruce, V. 2000. Do the eyes have it? Cues to the direction of social attention. *Trends in cognitive sciences,* 4**,** 50-59.

Larose, C., Richard-Yris, M. A., Hausberger, M. & Rogers, L. J. 2006. Laterality of horses associated with emotionality in novel situations. *Laterality,* 11**,** 355-67.

Leavens, D. A., Russell, J. L. & Hopkins, W. D. 2010. Multimodal communication by captive chimpanzees (Pan troglodytes). *Animal cognition,* 13**,** 33-40.

Ledoux, J. 2012. Rethinking the emotional brain. *Neuron,* 73**,** 653-76.

Lee, J., Floyd, T., Erb, H. & Houpt, K. 2011. Preference and demand for exercise in stabled horses. *Applied Animal Behaviour Science,* 130**,** 91-100.

Lee, T. W., Josephs, O., Dolan, R. J. & Critchley, H. D. 2006. Imitating expressions: emotion-specific neural substrates in facial mimicry. *Social Cognitive and Affective Neuroscience,* 1**,** 122-35.

Leiner, L. & Fendt, M. 2011. Behavioural fear and heart rate responses of horses after exposure to novel objects: Effects of habituation. *Applied Animal Behaviour Science,* 131**,** 104-109.

Lemasson, A., Boutin, A., Boivin, S., Blois-Heulin, C. & Hausberger, M. 2009. Horse (Equus caballus) whinnies: a source of social information. *Animal cognition,* 12**,** 693-704.

Liebal, K., Call, J., Tomasello, M. & Pika, S. 2004. To move or not to move: how apes adjust to the attentional state of others. *Interaction Studies,* 5**,** 199-219.

Liem, K. F. & Walker, W. F. 2001. *Functional anatomy of the vertebrates: an evolutionary perspective,* Harcourt College Publishers.

Likowski, K. U., Muhlberger, A., Gerdes, A. B., Wieser, M. J., Pauli, P. & Weyers, P. 2012. Facial mimicry and the mirror neuron system: simultaneous acquisition of facial electromyography and functional magnetic resonance imaging. *Frontiers in human neuroscience,* 6**,** 214.

Lima, S. L. & Bednekoff, P. A. 1999. Back to the basics of antipredatory vigilance: can nonvigilant animals detect attack? *Animal Behaviour,* 58**,** 537-543.

Lobmaier, J. S., Tiddeman, B. P. & Perrett, D. I. 2008. Emotional expression modulates perceived gaze direction. *Emotion,* 8**,** 573-7.

Ludwig, A., Pruvost, M., Reissmann, M., Benecke, N., Brockmann, G. A., Casta√±Os, P., Cieslak, M., Lippold, S., Llorente, L., Malaspinas, A.-S., Slatkin, M. & Hofreiter, M. 2009. Coat Color Variation at the Beginning of Horse Domestication. *Science,* 324**,** 485.

Mace, G. M., Harvey, P. H. & Clutton-Brock, T. H. 1981. Brain size and ecology in small mammals. *Journal of Zoology,* 193**,** 333-354.

Macuda, T. & Timney, B. 1999. Luminance and chromatic discrimination in the horse (Equus caballus). *Behavioural processes,* 44**,** 301-307.

Manser, M. B., Seyfarth, R. M. & Cheney, D. L. 2002. Suricate alarm calls signal predator class and urgency. *Trends in cognitive sciences,* 6**,** 55-57.

Marino, L. 2002. Convergence of complex cognitive abilities in cetaceans and primates. *Brain Behavior and Evolution,* 59**,** 21-32.

Maros, K., Gacsi, M. & Miklosi, A. 2008. Comprehension of human pointing gestures in horses (Equus caballus). *Animal cognition,* 11**,** 457-66.

Martin, R. D. 1981. Relative brain size and basal metabolic rate in terrestrial vertebrates. *Nature,* 293**,** 57-60.

Mayer, J. 2007. Use of behavior analysis to recognize pain in small mammals. *Lab Anim (NY),* 36**,** 43-8.

Mccomb, K., Moss, C., Durant, S. M., Baker, L. & Sayialel, S. 2001. Matriarchs as repositories of social knowledge in African elephants. *Science,* 292**,** 491-494.

Mccomb, K. & Semple, S. 2005. *Coevolution of vocal communication and sociality in primates*.

Mcdonnell, S. 2003. *The Equid Ethogram: A Practical Field Guide to Horse Behavior*, ECLIPSE Press.

Mcdonnell, S. M. & Haviland, J. C. S. 1995. Agonistic ethogram of the equid bachelor band.pdf. *Applied Animal Behaviour Science,* 43**,** 147 - 188.

Mcdonnell, S. M. & Poulin, A. 2002. Equid play ethogram. *Applied Animal Behaviour Science,* 78**,** 263-290.

Mcgreevy, P. D., Cripps, P. J., French, N. P., Green, L. E. & Nicol, C. J. 1995. Management factors associated with stereotypic and redirected behaviour in the Thoroughbred horse. *Equine Veterinary Journal,* 27**,** 86-91.

Mckinley, J. & Sambrook, T. D. 2000. Use of human-given cues by domestic dogs (Canis familiaris) and horses (Equus caballus). *Animal cognition,* 3**,** 13-22.

Mclean, A. N. 2004. Short-term spatial memory in the domestic horse. *Applied Animal Behaviour Science,* 85**,** 93-105.

Mendl, M., Burman, O. H. & Paul, E. S. 2010. An integrative and functional framework for the study of animal emotion and mood. *Proceedings. Biological sciences / The Royal Society,* 277**,** 2895-904.

Mendl, M. & Paul, E. 2004. Consciousness, emotion and animal welfare: Insights from cognitive science. *Animal Welfare,* 13**,** 17-25.

Micheletta, J. & Waller, B. M. 2012. Friendship affects gaze following in a tolerant species of macaque, Macaca nigra. *Animal Behaviour,* 83**,** 459-467.

Micheletta, J., Waller, B. M., Panggur, M. R., Neumann, C., Duboscq, J., Agil, M. & Engelhardt, A. 2012. Social bonds affect anti-predator behaviour in a tolerant species of macaque, Macaca nigra. *Proceedings. Biological sciences / The Royal Society,* 279**,** 4042-50.

Micheletta, J., Whitehouse, J., Parr, L. A. & Waller, B. M. 2015. Facial expression recognition in crested macaques (Macaca nigra). *Animal cognition,* 1-6.

Miklosi, Å. & Soproni, K. 2006. A comparative analysis of animals' understanding of the human pointing gesture. *Animal cognition,* 9**,** 81-93.

Miller, P. E. & Murphy, C. J. 1995. Vision in dogs. *J Am Vet Med Assoc,* 207**,** 1623-34.

Mills, D. S. & Nankervis, K. J. 2013. *Equine Behaviour: Principles and Practice*, Wiley.

Molony, V., Kent, J. E. & Mckendrick, I. J. 2002. Validation of a method for assessment of an acute pain in lambs. *Applied Animal Behaviour Science,* 76**,** 215-238.

Mulcahy, N. J. & Call, J. 2009. The performance of bonobos (Pan paniscus), chimpanzees (Pan troglodytes), and orangutans (Pongo pygmaeus) in two versions of an object-choice task. *Journal of Comparative Psychology,* 123**,** 304.

Murphy, E., Nordquist, R. E. & Van Der Staay, F. J. 2014. A review of behavioural methods to study emotion and mood in pigs, Sus scrofa. *Applied Animal Behaviour Science,* 159**,** 9-28.

Nakashima, S. F., Ukezono, M., Nishida, H., Sudo, R. & Takano, Y. 2015. Receiving of emotional signal of pain from conspecifics in laboratory rats. *Royal Society Open Science,* 2.

Nobre, A. C. & Kastner, S. 2014. *The Oxford Handbook of Attention,* OUP Oxford.

Nunez, C. M. V., Adelman, J. S. & Rubenstein, D. I. 2014. Sociality increases juvenile survival after a catastrophic event in the feral horse (Equus caballus). *Behavioral Ecology,* 26**,** 138-147.

O'callaghan, K. 2002. Lamneness and associated pain in cattle - challenging traditional perceptions. *In Practice,* 24**,** 212-219.

Okamoto-Barth, S., Call, J. & Tomasello, M. 2007. Great apes' understanding of other individuals' line of sight. *Psychological science,* 18**,** 462-468.

Orlando, L., Ginolhac, A., Zhang, G., Froese, D., Albrechtsen, A., Stiller, M., Schubert, M., Cappellini, E., Petersen, B., Moltke, I., Johnson, P. L. F., Fumagalli, M., Vilstrup, J. T., Raghavan, M., Korneliussen, T., Malaspinas, A.-S., Vogt, J., Szklarczyk, D., Kelstrup, C. D., Vinther, J., Dolocan, A., Stenderup, J., Velazquez, A. M. V., Cahill, J., Rasmussen, M., Wang, X., Min, J., Zazula, G. D., Seguin-Orlando, A., Mortensen, C., Magnussen, K., Thompson, J. F., Weinstock, J., Gregersen, K., Roed, K. H., Eisenmann, V., Rubin, C. J., Miller, D. C., Antczak, D. F., Bertelsen, M. F., Brunak, S., Al-Rasheid, K. a. S., Ryder, O., Andersson, L., Mundy, J., Krogh, A., Gilbert, M. T. P., Kjaer, K., Sicheritz-Ponten, T., Jensen, L. J., Olsen, J. V., Hofreiter, M., Nielsen, R., Shapiro, B., Wang, J. & Willerslev, E. 2013. Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse. *Nature,* 499**,** 74-78.

Osthaus, B., Proops, L., Hocking, I. & Burden, F. 2013. Spatial cognition and perseveration by horses, donkeys and mules in a simple A-not-B detour task. *Animal cognition,* 16**,** 301-5.

Outram, A. K., Stear, N. A., Bendrey, R., Olsen, S., Kasparov, A., Zaibert, V., Thorpe, N. & Evershed, R. P. 2009. The Earliest Horse Harnessing and Milking. *Science,* 323**,** 1332-1335.

Pack, A. A. & Herman, L. M. 2007. The dolphin's (Tursiops truncatus) understanding of human gazing and pointing: knowing what and where. *Journal of Comparative Psychology,* 121**,** 34-45.

Panksepp, J. 1998. *Affective Neuroscience: The Foundations of Human and Animal Emotions,* Oxford University Press.

Panksepp, J. 2005a. Affective consciousness: Core emotional feelings in animals and humans. *Consciousness and Cognition,* 14**,** 30-80.

Panksepp, J. 2005b. Psychology. Beyond a joke: from animal laughter to human joy? *Science,* 308**,** 62-3.

Panksepp, J. & Burgdorf, J. 2003. "Laughing" rats and the evolutionary antecedents of human joy? *Physiology & Behavior,* 79**,** 533-547.

Parent, A. 2005. Duchenne De Boulogne: a pioneer in neurology and medical photography. *Can J Neurol Sci,* 32**,** 369-77.

Parr, L. A. 2001. Cognitive and physiological markers of emotional awareness in chimpanzees (Pan troglodytes). *Animal cognition,* 4**,** 223-229.

Parr, L. A. 2003. The Discrimination of Faces and Their Emotional Content by Chimpanzees (Pan troglodytes). *Annals of the New York Academy of Sciences,* 1000**,** 56-78.

Parr, L. A. 2011. The evolution of face processing in primates. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 366**,** 1764-77.

Parr, L. A., Cohen, M. & Waal, F. 2005. Influence of Social Context on the Use of Blended and Graded Facial Displays in Chimpanzees. *International Journal of Primatology,* 26**,** 73-103.

Parr, L. A. & Heintz, M. 2009. Facial expression recognition in rhesus monkeys, Macaca mulatta. *Animal Behaviour,* 77**,** 1507-1513.

Parr, L. A. & Hopkins, W. D. 2001. Brain temperature asymmetries and emotional perception in chimpanzees, Pan troglodytes. *Physiology & Behavior,* 71**,** 363-371.

Parr, L. A., Hopkins, W. D. & De Waal, F. 1998. The perception of facial expressions by chimpanzees, Pan troglodytes. *Evolution of Communication,* 2**,** 1-23.

Parr, L. A., Waller, B. M., Burrows, A. M., Gothard, K. M. & Vick, S. J. 2010. MaqFACS: A muscle-based facial movement coding system for the rhesus macaque. *American journal of physical anthropology,* 143**,** 625-30.

Parr, L. A., Waller, B. M., Vick, S. J. & Bard, K. A. 2007. Classifying chimpanzee facial expressions using muscle action. *Emotion,* 7**,** 172-81.

Partan, S. R., Larco, C. P. & Owens, M. J. 2009. Wild tree squirrels respond with multisensory enhancement to conspecific robot alarm behaviour. *Animal Behaviour,* 77**,** 1127-1135.

Peignot, P. & Anderson, J. R. 1999. Use of experimenter-given manual and facial cues by gorillas (Gorilla gorilla) in an object-choice task. *Journal of Comparative Psychology,* 113**,** 253.

Pellis, S. M. & Pellis, V. C. 1997. Targets, tactics, and the open mouth face during play fighting in three species of primates. *Aggressive Behavior,* 23**,** 41-57.

Pérez-Barbería, F. J., Shultz, S. & Dunbar, R. I. M. 2007. Evidence for coevolution of sociality and relative brain size in three orders of mammals. *Evolution,* 61**,** 2811-2821.

Perez-Barberria, F. J. & Gordon, I. J. 2005. Gregariousness increases brain size in ungulates. *Oecologia,* 145**,** 41-52.

Perrett, D., Smith, P., Potter, D., Mistlin, A., Head, A., Milner, A. & Jeeves, M. 1985. Visual cells in the temporal cortex sensitive to face view and gaze direction. *Proceedings of the Royal society of London. Series B. Biological sciences,* 223**,** 293-317.

Peters, G. 2002. Purring and similar vocalizations in mammals. *Mammal Review,* 32**,** 245-271.

Pika, S., Liebal, K. & Tomasello, M. 2005. Gestural communication in subadult bonobos (Pan paniscus): Repertoire and use. *American Journal of Primatology,* 65**,** 39-61.

Piva, E., Liverani, V., Accorsi, P. A., Sarli, G. & Gandini, G. 2008. Welfare in a shelter dog rehomed with Alzheimer patients. *Journal of Veterinary Behavior: Clinical Applications and Research,* 3**,** 87-94.

Pond, R. L., Darre, M. J., Scheifele, P. M. & Browning, D. G. 2010. Characterization of equine vocalization. *Journal of Veterinary Behavior: Clinical Applications and Research,* 5**,** 7-12.

Poole, T. B. 1978. Analysis of social play in polecats (*Mustelidae*) with comments on form and evolutionary history of open mouth play face. *Animal Behaviour,* 26**,** 36-49.

Povinelli, D. J. & Eddy, T. J. 1996a. Chimpanzees: joint visual attention. *Psychological science,* 7**,** 129-135.

Povinelli, D. J. & Eddy, T. J. 1996b. Factors influencing young chimpanzees' (Pan troglodytes) recognition of attention. *Journal of Comparative Psychology,* 110**,** 336-345.

Povinelli, D. J. & Eddy, T. J. 1997. Specificity of gaze-following in young chimpanzees. *British Journal of Developmental Psychology,* 15**,** 213-222.

Povinelli, D. J., Eddy, T. J., Hobson, R. P. & Tomasello, M. 1996. What young chimpanzees know about seeing. *Monographs of the society for research in child development***,** i-189.

Pöysä, H. 1992. Group foraging in patchy environments: the importance of coarse-level local enhancement. *Ornis scandinavica***,** 159-166.

Premack, D. & Woodruff, G. 1978. Does the chimpanzee have a theory of mind? *Behavioral and Brain Sciences,* 1**,** 515-526.

Preuschoft, S. & Van Hooff, J. A. 1997. The social function of" smile" and" laughter": Variations across primate species and societies.

Prince, J. H., Diesem, C. D., Eglitis, I. & Ruskell, G. L. 1960. *Anatomy and Histology of the Eye and Orbit in Domestic Animals.* , Springfield, IL, Charles C. Thomas.

Proctor, H. S. & Carder, G. 2014. Can ear postures reliably measure the positive emotional state of cows? *Applied Animal Behaviour Science,* 161**,** 20-27.

Proops, L. & Mccomb, K. 2010. Attributing attention: the use of human-given cues by domestic horses (Equus caballus). *Animal cognition,* 13**,** 197-205.

Proops, L. & Mccomb, K. 2012. Cross-modal individual recognition in domestic horses (Equus caballus) extends to familiar humans. *Proceedings. Biological sciences / The Royal Society*.

Proops, L., Mccomb, K. & Reby, D. 2009. Cross-modal individual recognition in domestic horses (Equus caballus). *Proceedings of the National Academy of Sciences of the United States of America,* 106**,** 947-51.

Proops, L., Rayner, J., Taylor, A. M. & Mccomb, K. 2013. The Responses of Young Domestic Horses to Human-Given Cues. *PLoS one,* 8**,** e67000.

Proops, L., Walton, M. & Mccomb, K. 2010. The use of human-given cues by domestic horses, Equus caballus, during an object choice task. *Animal Behaviour,* 79**,** 1205-1209.

Pusey, A. & Packer, C. 2003. The Ecology of Relationships. *In:* Krebs, J. & Davies, N. (eds.) *Behavioural Ecology.* Oxford: Blackwell Scientific Publication.

Racca, A., Guo, K., Meints, K. & Mills, D. S. 2012. Reading faces: differential lateral gaze bias in processing canine and human facial expressions in dogs and 4-year-old children. *PLoS one,* 7**,** e36076.

Ransom, J. I. & Cade, B. S. 2009. Quantifying Equid Behaviour - A research ethogram for free roaming feral horses. Publications of the US Geological Survey.

Reaux, J. E., Theall, L. A. & Povinelli, D. J. 1999. A Longitudinal Investigation of Chimpanzees' Understanding of Visual Perception. *Child Development,* 70**,** 275-290.

Reed, T. & Brown, M. 2000. The expression of care in the rough and tumble play of boys. *Journal of Research in Childhood Education,* 15**,** 104-116.

Reefmann, N., Bütikofer Kaszàs, F., Wechsler, B. & Gygax, L. 2009a. Ear and tail postures as indicators of emotional valence in sheep. *Applied Animal Behaviour Science,* 118**,** 199-207.

Reefmann, N., Wechsler, B. & Gygax, L. 2009b. Behavioural and physiological assessment of positive and negative emotion in sheep. *Animal Behaviour,* 78**,** 651-659.

Reimert, I., Bolhuis, J. E., Kemp, B. & Rodenburg, T. B. 2013. Indicators of positive and negative emotions and emotional contagion in pigs. *Physiology & Behavior,* 109**,** 42-50.

Rhodin, M., Álvarez, C. B. G., Byström, A., Johnston, C., Van Weeren, P. R., Roepstorff, L. & Weishaupt, M. A. 2009. The effect of different head and neck positions on the caudal back and hindlimb kinematics in the elite dressage horse at trot. *Equine Veterinary Journal,* 41**,** 274-279.

Rinn, W. E. 1984. The neuropsychology of facial expression: A review of the neurological and psychological mechanisms for producing facial expressions. *Psychological Bulletin,* 95**,** 52-77.

Rivera, E., Benjamin, S., Nielsen, B., Shelle, J. & Zanella, A. J. 2002. Behavioral and physiological responses of horses to initial training: the comparison between pastured versus stalled horses. *Applied Animal Behaviour Science,* 78**,** 235-252.

Rosati, A. G. & Hare, B. 2009. Looking past the model species: diversity in gaze-following skills across primates. *Current opinion in neurobiology,* 19**,** 45-51.

Roth, L. S. V., Balkenius, A. & Kelber, A. 2008. The Absolute Threshold of Colour Vision in the Horse. *PLoS one,* 3**,** e3711.

Rubenstein, D. & Hack, M. A. 1992. Horse signals: the sounds and scents of fury. *Evolutionary Ecology,* 6**,** 245-260.

Rubenstein, D. I. 1981. Behavioural ecology of island feral horses. *Equine Veterinary Journal,* 13**,** 27-34.

Ruffman, T., Henry, J. D., Livingstone, V. & Phillips, L. H. 2008. A meta-analytic review of emotion recognition and aging: Implications for neuropsychological models of aging. *Neuroscience & Biobehavioral Reviews,* 32**,** 863-881.

Ruiz, A., Gómez, J., Roeder, J. & Byrne, R. 2009. Gaze following and gaze priming in lemurs. *Animal cognition,* 12**,** 427-434.

Russell, J. A. 2003. Core affect and the psychological construction of emotion. *Psychological Review,* 110**,** 145.

Sandem, A. I. & Braastad, B. O. 2005. Effects of cow–calf separation on visible eye white and behaviour in dairy cows—A brief report. *Applied Animal Behaviour Science,* 95**,** 233-239.

Sandem, A. I., Braastad, B. O. & Bøe, K. E. 2002. Eye white may indicate emotional state on a frustration-contentedness axis in dairy cows. *Applied Animal Behaviour Science,* 79**,** 1-10.

Sandem, A. I., Janczak, A. M. & Braastad, B. O. 2004. A short note on effects of exposure to a novel stimulus (umbrella) on behaviour and percentage of eye-white in cows. *Applied Animal Behaviour Science,* 89**,** 309-314.

Sandem, A. I., Janczak, A. M., Salte, R. & Braastad, B. O. 2006. The use of diazepam as a pharmacological validation of eye white as an indicator of emotional state in dairy cows. *Applied Animal Behaviour Science,* 96**,** 177-183.

Sankey, C., Richard-Yris, M.-A., Henry, S. V., Fureix, C., Nassur, F. & Hausberger, M. 2010a. Reinforcement as a mediator of the perception of humans by horses (Equus caballus). *Animal cognition,* 13**,** 753-764.

Sankey, C., Richard-Yris, M.-A., Leroy, H., Henry, S. & Hausberger, M. 2010b. Positive interactions lead to lasting positive memories in horses, Equus caballus. *Animal Behaviour,* 79**,** 869-875.

Sapolsky, R. M. 2007. *A Primate's Memoir: A Neuroscientist's Unconventional Life Among the Baboons*, Scribner.

Sappington, B. & Goldman, L. 1994. Discrimination learning and concept formation in the Arabian horse. *Journal of Animal Science,* 72**,** 3080-3087.

Saslow, C. A. 1999. Factors affecting stimulus visibility for horses. *Applied Animal Behaviour Science,* 61**,** 273-284.

Saslow, C. A. 2002. Understanding the perceptual world of horses. *Applied Animal Behaviour Science,* 78**,** 209-224.

Sato, N., Tan, L., Tate, K. & Okada, M. 2015. Rats demonstrate helping behavior toward a soaked conspecific. *Animal cognition***,** 1-9.

Sauter, D. A., Eisner, F., Ekman, P. & Scott, S. K. 2010. Cross-cultural recognition of basic emotions through nonverbal emotional vocalizations. *Proceedings of the National Academy of Sciences of the United States of America,* 107**,** 2408-12.

Schel, A. M., Townsend, S. W., Machanda, Z., Zuberbühler, K. & Slocombe, K. E. 2013. Chimpanzee alarm call production meets key criteria for intentionality. *PLoS one,* 8**,** e76674.

Scheumann, M., Zimmermann, E. & Deichsel, G. 2007. Context-specific calls signal infants' needs in a strepsirrhine primate, the gray mouse lemur (Microcebus murinus). *Developmental psychobiology,* 49**,** 708-18.

Schilder, M. B. H., Van Hooff, J. a. R. a. M., Van Geer-Plesman, C. J. & Wensing, J. B. 1984. A Quantitative Analysis of Facial Expression in the Plains Zebra. *Zeitschrift für Tierpsychologie,* 66**,** 11-32.

Schmidt, K. L. & Cohn, J. F. 2001. Human facial expressions as adaptations: Evolutionary questions in facial expression research. *American journal of physical anthropology,* Suppl 33**,** 3-24.

Schneider, G. & Krueger, K. 2012. Third-party interventions keep social partners from exchanging affiliative interactions with others. *Animal Behaviour,* 83**,** 377-387.

Seyfarth, R. M. & Cheney, D. L. 2003. Signalers and receivers in animal communication. *Annual Review of Psychology,* 54**,** 145-73.

Seyfarth, R. M., Cheney, D. L., Bergman, T. J., Fischer, J., Zuberbühler, K. & Hammerschmidt, K. 2010. The central importance of information in studies of animal communication. *Animal Behaviour,* 80**,** 3-8.

Shepherd, S. V., Deaner, R. O. & Platt, M. L. 2006. Social status gates social attention in monkeys. *Current Biology,* 16**,** R119-R120.

Shepherd, S. V. & Platt, M. L. 2008. Spontaneous social orienting and gaze following in ringtailed lemurs (Lemur catta). *Animal cognition,* 11**,** 13-20.

Shultz, S. & Dunbar, R. 2010. Encephalization is not a universal macroevolutionary phenomenon in mammals but is associated with sociality. *Proceedings of the National Academy of Sciences,* 107**,** 21582-21586.

Shultz, S. & Dunbar, R. I. 2006. Both social and ecological factors predict ungulate brain size. *Proceedings. Biological sciences / The Royal Society,* 273**,** 207-15.

Silk, J. B., Beehner, J. C., Bergman, T. J., Crockford, C., Engh, A. L., Moscovice, L. R., Wittig, R. M., Seyfarth, R. M. & Cheney, D. L. 2009. The benefits of social capital: close social bonds among female baboons enhance offspring survival. *Proceedings. Biological sciences / The Royal Society,* 276**,** 3099-104.

Silk, J. B., Beehner, J. C., Bergman, T. J., Crockford, C., Engh, A. L., Moscovice, L. R., Wittig, R. M., Seyfarth, R. M. & Cheney, D. L. 2010. Strong and consistent

social bonds enhance the longevity of female baboons. *Current biology : CB,* 20**,** 1359-61.

Slocombe, K. E., Townsend, S. W. & Zuberbuhler, K. 2009. Wild chimpanzees (Pan troglodytes schweinfurthii) distinguish between different scream types: evidence from a playback study. *Animal cognition,* 12**,** 441-9.

Slocombe, K. E., Waller, B. M. & Liebal, K. 2011. The language void: the need for multimodality in primate communication research. *Animal Behaviour,* 81**,** 919-924.

Slocombe, K. E. & Zuberbühler, K. 2007. Chimpanzees modify recruitment screams as a function of audience composition. *Proceedings of the National Academy of Sciences,* 104**,** 17228-17233.

Smet, A. F. & Byrne, R. W. 2013. African elephants can use human pointing cues to find hidden food. *Current Biology,* 23**,** 2033-2037.

Smet, A. F. & Byrne, R. W. 2014a. African elephants (Loxodonta africana) recognize visual attention from face and body orientation. *Biology Letters,* 10**,** 20140428.

Smet, A. F. & Byrne, R. W. 2014b. Interpretation of human pointing by African elephants: generalisation and rationality. *Animal cognition,* 17**,** 1365-1374.

Søndergaard, E. & Ladewig, J. 2004. Group housing exerts a positive effect on the behaviour of young horses during training. *Applied Animal Behaviour Science,* 87**,** 105-118.

Soproni, K., Miklósi, Å., Topál, J. & Csányi, V. 2001. Comprehension of human communicative signs in pet dogs (Canis familiaris). *Journal of Comparative Psychology,* 115**,** 122.

Sotocinal, S. G., Sorge, R. E., Zaloum, A., Tuttle, A. H., Martin, L. J., Wieskopf, J. S., Mapplebeck, J. C., Wei, P., Zhan, S., Zhang, S., Mcdougall, J. J., King, O. D. & Mogil, J. S. 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular pain,* 7**,** 55.

Stoddart, D. M. 1980. *Ecology of Vertebrate Olfaction,* Berlin, Springer Science & Business Media.

Stone, S. M. 2010. Human facial discrimination in horses: can they tell us apart? *Animal cognition,* 13**,** 51-61.

Sundaresan, S. R., Fischhoff, I. R., Dushoff, J. & Rubenstein, D. I. 2007. Network metrics reveal differences in social organization between two fission-fusion species, Grevy's zebra and onager. *Oecologia,* 151**,** 140-9.

Tabor, R. 2003. *Understanding Cat Behavior*, F+W Media.

Tate, A. J., Fischer, H., Leigh, A. E. & Kendrick, K. M. 2006. Behavioural and neurophysiological evidence for face identity and face emotion processing in animals. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 361**,** 2155-72.

Taylor, A. M. & Reby, D. 2010. The contribution of source-filter theory to mammal vocal communication research. *Journal of Zoology,* 280**,** 221-236.

Tempelmann, S., Kaminski, J. & Liebal, K. 2011. Focus on the essential: all great apes know when others are being attentive. *Animal cognition,* 14**,** 433-439.

Teufel, C., Gutmann, A., Pirow, R. & Fischer, J. 2010. Facial expressions modulate the ontogenetic trajectory of gaze-following among monkeys. *Developmental science,* 13**,** 913-922.

Timney, B. & Keil, K. 1992. Visual acuity in the horse. *Vision Res,* 32**,** 2289-93.

Timney, B. & Keil, K. 1999. Local and global stereopsis in the horse. *Vision Research,* 39**,** 1861-1867.

Tomasello, M., Call, J. & Hare, B. 1998. Five primate species follow the visual gaze of conspecifics. *Animal Behaviour,* 55**,** 1063-1069.

Tomasello, M., Hare, B. & Fogleman, T. 2001. The ontogeny of gaze following in chimpanzees, Pan troglodytes, and rhesus macaques, Macaca mulatta. *Animal Behaviour,* 61**,** 335-343.

Tomasello, M., Hare, B., Lehmann, H. & Call, J. 2007a. Reliance on head versus eyes in the gaze following of great apes and human infants: the cooperative eye hypothesis. *Journal of human evolution,* 52**,** 314-320.

Tomasello, M., Hare, B., Lehmann, H. & Call, J. 2007b. Reliance on head versus eyes in the gaze following of great apes and human infants: the cooperative eye hypothesis. *Journal of human evolution,* 52**,** 314-20.

Tschudin, A., Call, J., Dunbar, R. I., Harris, G. & Van Der Elst, C. 2001. Comprehension of signs by dolphins (Tursiops truncatus). *Journal of Comparative Psychology,* 115**,** 100.

Tyler, S. J. 1972. The behaviour and social organisation of the new forest ponies. *Animal Behaviour Monographs,* 5**,** 87-196.

Valone, T. J. 1989. Group foraging, public information, and patch estimation. *Oikos,* 357-363.

Valone, T. J. & Templeton, J. J. 2002. Public information for the assessment of quality: a widespread social phenomenon. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 357**,** 1549-1557.

Van Hooff, J. a. R. a. M. 1972. A comparative approach to the phylogeny of laughter and smiling. *In:* Hinde, R. A. (ed.) *Non-Verbal Communication.* Cambridge, UK: Cambridge University Press.

Veissier, I., Boissy, A., Désiré, L. & Greiveldinger, L. 2009. Animals' emotions: studies in sheep using appraisal theories. *Animal Welfare,* 18**,** 347-54.

Vick, S. J. & Anderson, J. R. 2000. Learning and limits of use of eye gaze by capuchin monkeys (Cebus apella) in an object-choice task. *Journal of Comparative Psychology,* 114**,** 200.

Vick, S. J., Toxopeus, I. & Anderson, J. R. 2006. Pictorial gaze cues do not enhance long-tailed macaques performance on a computerised object-location task. *Behavioural processes,* 73**,** 308-314.

Vick, S. J., Waller, B. M., Parr, L. A., Smith Pasqualini, M. C. & Bard, K. A. 2007. A Cross-species Comparison of Facial Morphology and Movement in Humans and Chimpanzees Using the Facial Action Coding System (FACS). *Journal of nonverbal behavior,* 31**,** 1-20.

Virányi, Z., Gácsi, M., Kubinyi, E., Topál, J., Belényi, B., Ujfalussy, D. & Miklósi, Å. 2008. Comprehension of human pointing gestures in young human-reared wolves (Canis lupus) and dogs (Canis familiaris). *Animal cognition,* 11**,** 373-387.

Von Borell, E., Langbein, J., Despres, G., Hansen, S., Leterrier, C., Marchant-Forde, J., Marchant-Forde, R., Minero, M., Mohr, E., Prunier, A., Valance, D. & Veissier, I. 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals -- a review. *Physiology & Behavior,* 92**,** 293-316.

Von Grünau, M. & Anston, C. 1995. The detection of gaze: a stare in the crowd effect. *Perception,* 24**,** 1297-1313.

Wall, S. B. V. 1990. *Food hoarding in animals,* University of Chicago Press.

Waller, B. M., Bard, K. A., Vick, S. J. & Smith Pasqualini, M. C. 2007. Perceived differences between chimpanzee (Pan troglodytes) and human (Homo sapiens)

facial expressions are related to emotional interpretation. *Journal of Comparative Psychology,* 121**,** 398-404.

Waller, B. M., Caeiro, C. C. & Davila-Ross, M. 2015. Orangutans modify facial displays depending on recipient attention. *PeerJ,* 3**,** e827.

Waller, B. M., Cray, J. J. & Burrows, A. M. 2008a. Selection for universal facial emotion. *Emotion,* 8**,** 435-9.

Waller, B. M. & Dunbar, R. I. 2005. Differential behavioural effects of silent bared teeth display and relaxed open mouth display in chimpanzees. *Ethology,* 111**,** 129-142.

Waller, B. M. & Micheletta, J. 2013. Facial Expression in Nonhuman Animals. *Emotion Review,* 5**,** 54-59.

Waller, B. M., Misch, A., Whitehouse, J. & Herrmann, E. 2014. Children, but not chimpanzees, have facial correlates of determination. *Biology letters,* 10**,** 20130974.

Waller, B. M., Parr, L. A., Gothard, K. M., Burrows, A. M. & Fuglevand, A. J. 2008b. Mapping the contribution of single muscles to facial movements in the rhesus macaque. *Physiology & Behavior,* 95**,** 93-100.

Waller, B. M., Peirce, K., Caeiro, C. T. C., Scheider, L., Burrows, A. M., Mccune, S. & Kaminski, J. 2013. Paedomorphic Facial Expressions Give Dogs a Selective Advantage. *PLoS one,* 8**,** e82686.

Waran, N. K. 1997. Can studies of feral horse behaviour be used for assessing domestic horse welfare? *Equine Veterinary Journal,* 29**,** 249-251.

Waring, G. H. 2003. *Horse Behavior,* Noyes Publications/William Andrew Pub.

Warmuth, V., Eriksson, A., Bower, M. A., Barker, G., Barrett, E., Hanks, B. K., Li, S., Lomitashvili, D., Ochir-Goryaeva, M., Sizonov, G. V., Soyonov, V. & Manica, A. 2012. Reconstructing the origin and spread of horse domestication in the Eurasian steppe. *Proceedings of the National Academy of Sciences,* 109**,** 8202-8206.

Warren, J. & Warren, H. B. 1962. Reversal learning by horse and raccoon. *The Journal of genetic psychology,* 100**,** 215-220.

Warren, J. E., Sauter, D. A., Eisner, F., Wiland, J., Dresner, M. A., Wise, R. J., Rosen, S. & Scott, S. K. 2006. Positive emotions preferentially engage an auditory-motor "mirror" system. *The Journal of neuroscience,* 26**,** 13067-75.

Weeks, J. W., Crowell-Davis, S. L., Caudle, A. B. & Heusner, G. L. 2000. Aggression and social spacing in light horse (Equus caballus) mares and foals. *Applied Animal Behaviour Science,* 68**,** 319-337.

Weishaupt, M. A., Wiestner, T., Von Peinen, K., Waldern, N., Roepstorff, L., Van Weeren, R., Meyer, H. & Johnston, C. 2006. Effect of head and neck position on vertical ground reaction forces and interlimb coordination in the dressage horse ridden at walk and trot on a treadmill. *Equine Veterinary Journal,* 38**,** 387-392.

Whalen, P. J., Kagan, J., Cook, R. G., Davis, F. C., Kim, H., Polis, S., Mclaren, D. G., Somerville, L. H., Mclean, A. A., Maxwell, J. S. & Johnstone, T. 2004. Human Amygdala Responsivity to Masked Fearful Eye Whites. *Science,* 306**,** 2061.

White, D. J. & Galef, B. G., Jr. 2000. Differences between the sexes in direction and duration of response to seeing a potential sex partner mate with another. *Animal Behaviour,* 59**,** 1235-1240.

Whitehead, H. & Rendell, L. 2014. *The Cultural Lives of Whales and Dolphins,* University of Chicago Press.

Whitehead, R. D., Re, D., Xiao, D., Ozakinci, G. & Perrett, D. I. 2012. You are what you eat: within-subject increases in fruit and vegetable consumption confer beneficial skin-color changes. *PLoS one,* 7**,** e32988.

Whiten, A. & Byrne, R. W. 1988. Tactical deception in primates. *Behavioral and Brain Sciences,* 11**,** 233-244.

Whiten, A. & Byrne, R. W. 1989. The manipulation of attention in primate tactical deception. *In:* Byrne, R. W. & Whiten, A. (eds.) *Machiavellian Intelligence: Social Expertise And The Evolution Of Intellect In Monkeys, Apes, And Humans* Oxford, UK: Oxford University Press.

Whiten, A. & Van Schaik, C. P. 2007. The evolution of animal 'cultures' and social intelligence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 362**,** 603-20.

Wilkinson, A., Mandl, I., Bugnyar, T. & Huber, L. 2010. Gaze following in the red-footed tortoise (Geochelone carbonaria). *Animal cognition,* 13**,** 765-769.

Williams, A. C. D. C. 2002. Facial expression of pain, empathy, evolution, and social learning. *Behavioral and Brain Sciences,* 25**,** 475-480.

Wohr, M. & Schwarting, R. K. 2007. Ultrasonic communication in rats: can playback of 50-kHz calls induce approach behavior? *PLoS one,* 2**,** e1365.

Wolski, T. R., Houpt, K. A. & Aronson, R. 1980. The role of the senses in mare foal recognition. *Applied Animal Ethology,* 6**,** 121-138.

Yeon, S. C. 2012. Acoustic communication in the domestic horse (Equus caballus). *Journal of Veterinary Behavior: Clinical Applications and Research,* 7**,** 179-185.

Yeon, S. C., Kim, Y. K., Park, S. J., Lee, S. S., Lee, S. Y., Suh, E. H., Houpt, K. A., Chang, H. H., Lee, H. C., Yang, B. G. & Lee, H. J. 2011. Differences between vocalization evoked by social stimuli in feral cats and house cats. *Behavioural processes,* 87**,** 183-189.

Yin, S. & Mccowan, B. 2004. Barking in domestic dogs: context specificity and individual identification. *Animal Behaviour,* 68**,** 343-355.

Article I

EquiFACS: The Equine Facial Action Coding System

Wathan, J. Burrows, A.M., Waller, B.M., & McComb, K. (2015). *PLOS ONE*, in press.

Abstract

Although previous studies of horses have investigated their facial expressions in specific contexts, e.g. pain [1, 2], until now there has been no methodology available that documents all the possible facial movements of the horse and provides a way to record all potential facial configurations. This is essential for an objective description of horse facial expressions across a range of contexts that reflect different emotional states. Facial Action Coding Systems (FACS) provide a systematic methodology of identifying and coding facial expressions on the basis of underlying facial musculature and muscle movement. FACS are anatomically based and document all possible facial movements rather than a configuration of movements associated with a particular situation. Consequently, FACS can be applied as a tool for a wide range of research questions. We developed FACS for the domestic horse (*Equus caballus*) through anatomical investigation of the underlying musculature and subsequent analysis of naturally occurring behaviour captured on high quality video. Discrete facial movements were identified and described in terms of the underlying muscle contractions, in correspondence with previous FACS systems. The reliability of others to be able to learn this system (EquiFACS) and consistently code behavioural sequences was high - and this included people with no previous experience of horses. A wide range of facial movements were identified, including many that are also seen in primates and other domestic animals (dogs and cats). EquiFACS provides a method that can now be used to document the facial movements associated with different social contexts and thus to address questions relevant to understanding social cognition and comparative psychology, as well as informing current veterinary and animal welfare practices.

Introduction

Facial Action Coding Systems (FACS) provide a method of identifying and recording facial expressions based on the underlying facial (mimetic) musculature and muscle movement [3]. Here we present EquiFACS, a Facial Action Coding System for the domestic horse (*Equus caballus*). Until now, there has been no methodology available that documented all of the facial movements of the horse, allowing a record of all potential facial configurations. There are a number of studies that focus on the facial expressions of horses in one specific context, for example pain [1, 2]. However, EquiFACS provides a comprehensive list of all the facial movements that horses can produce, which can be used to document facial expressions across a wide range of contexts.

What are Facial Action Coding Systems?

FACS are objective coding systems for describing facial behaviour. The facial muscles (sometimes known as the mimetic muscles) are a subgroup of muscles innervated by CN7 (the facial nerve). They are characterised by their extensive connections to the superficial fascia and skin of the face, and consequently are responsible for observable changes in the skin (facial movements or expressions) [4]. FACS are frameworks where designated codes (Action Units or AUs) represent the contraction of a particular facial muscle (or set of muscles) and the resulting facial movements [3]. Action Descriptors (ADs) are also used for more general facial movements where the muscular basis either cannot be identified or is the result of a different muscle set (e.g. deep muscles). This creates a reliable system that people can be trained to use and that describes facial actions in a standardised way, avoiding subjective assessments of expression. This is particularly important as the recording and analysis of facial expressions can be subject to a large degree of observer bias and influenced by the perceived emotional context [5-7]. Consequently, frameworks that quantify behaviour and allow reliable, objective measurements are needed.

The original FACS was developed for use in humans [3] and this framework has since been applied to a number of different primates and domestic animals (chimpanzees (*Pan troglodytes*)[8], orangutans (*Pongo pygmaeus*)[9], rhesus macaques (*Macaca mulatta*)[10], *hylobatids* (gibbons and siamangs)[11], dogs (*Canis familiaris*)[12] and

cats (*Felis catus*)[13]. This methodology provides compatible systems that allow direct comparisons using identical techniques across species with a different facial morphology (e.g. [14]). EquiFACS is the first attempt to develop this system for an animal with laterally placed eyes and an elongated face.

Why are horses an interesting model?

Horses belong to the order Perissodactyla, and within this they are one of nine species in the family Equus. By the end of the Pleistocene era (approx. 2.5 million – 12,000 years ago) wild horses (*Equus ferus*) roamed across Europe, Asia and North America, and it is estimated that the domestication of horses began around 5000 years ago [15]. However, by the middle of the twentieth century horses had become extinct in the wild, yet were thriving as a domestic species [16]. Domestication can dramatically influence the social, cognitive, and morphological characteristics of a species, and the study of domestic species is of great interest from both welfare and evolutionary perspectives [17-19].

Horses are long-lived social animals. Feral populations have demonstrated that without domestic pressures horses would live in a society comprising of several small groups or 'bands' that share space and resources, and to which membership stays relatively stable over time. Bands have large, overlapping ranges so horses regularly come into contact with many other conspecifics, and inter-band dominance indicates that within the larger herd established social relationships exist [20]. Consequently, horses show fission-fusion dynamics; a variation of the same complex social organisation that is seen in humans, bonobos, chimpanzees, and macaques, as well as elephants, spotted hyenas and many cetaceans [21]. Group life in these societies is determined by complex, long-term social relationships that must be maintained, suggesting effective communication would be adaptive [22].

Horses are predominantly visual animals, with reasonable visual acuity that, at 23 cycles per degree, is better than domestic cats and dogs [23-25]. While horses' use of head and body posture in signaling has been described in observational literature (e.g. [20] and [26]), surprisingly their use of facial expressions has been largely overlooked. This is despite attempts to quantify facial expressions in horses' close relatives, plains

zebra (*Equus quagga* [27]), and reports that horses do routinely use some apparently complex facial expressions (e.g. snapping and the estrous face, which both involve pulling back the lips and flattening of the ears [28]).

A systematic way of recording facial expressions would have a wide range of uses, with the potential to assess and improve welfare for horses, as well as enhancing our understanding of communication and cognition in this highly social species and providing insights into the effects of domestication. Questions about whether particular facial movements are associated with negative emotional states or indicative of positive experiences will be particularly important to address, and FACS provides an ideal framework for such investigations. More generally, FACS provides a framework through which species with different phylogenetic and ecological influences can be compared to investigate the functional significance and evolutionary origins of facial expressions.

How was FACS modified for use in horses?

To modify FACS for use in non-human animals, the first step was to document and compare their facial anatomy [9-13, 29]. Horses are only distantly related to primates and the other animals FACS have previously been developed for; additionally horses have a dramatically different facial morphology to these animals, including laterally placed eyes, elongated faces, and raised zygomatic arches. Consequently, it is possible that the facial muscles of horses would show little similarity with the facial muscles of humans or those underpinning other FACS.

To facilitate valid comparisons across taxa, it is necessary to report findings in a consistent way, with a standardised nomenclature for the facial muscles [4]. While the anatomy of domestic mammals is generally well documented, it has been difficult to make direct comparisons between domestic animals and primates because of the inconsistent terminology used (see [4, 30] for reviews and a call for a standardized nomenclature for the facial muscles). Additionally, the anatomy of domestic mammals tends to be described from a veterinary perspective, so that the muscles of facial expression are often not documented comprehensively within one text (e.g. [31]). Therefore, we supplemented the previous literature on the facial anatomy of the horse

with our own dissection using an innovative ‘face mask’ method, which allows a more complete view of the facial muscles and their attachments than traditional dissection methods [32-35]. In doing this we documented the muscles using the recommended terms, which allowed us to make direct comparisons between horses and the other species. The facial muscles of the horse are reported here using the standardised terminology [4], so that a free, detailed record of the muscles of facial expression is available alongside the EquiFACS manual.

We then analysed footage of a wide range of naturally occurring horse behaviours captured on high quality video. Discrete facial movements were identified, their proposed muscular basis was noted, and they were given a code in correspondence with previous FACS systems. All the facial movements identified are listed in the results, accompanied by a description of the appearance changes and video illustrations. Where necessary, the proposed muscular basis of the actions is given and there are also sections on subtle differences between similar actions.

Method

Anatomical investigation of the underlying anatomy

The head of one horse was acquired from the New Bolton Centre at the University of Pennsylvania. The dissection was done using an innovative ‘face mask’ method, where the facial musculature is removed from the deep muscles and the skull with the skin, creating a ‘facial mask’ that holds all of the facial muscles (see S1 Text for full details of the dissection procedure). Using this novel approach preserves the superficially located facial muscles that might be lost in the traditional dissection method of removing the skin from the facial musculature. Furthermore, it provides a more complete picture of muscle attachments, by keeping superficial portions attached to the skin and deeper portions attached to the skull (see [32-35]). The muscles were examined for presence/absence, attachments to the bone, skin, and cartilage, as well as their three-dimensional relationships to one another and to the skull. Muscles were classified with reference to a variety of sources (mainly [36, 37]) and in relation to previous dissections complete for the development of FACS in other species [32-35]. All muscles and their attachments were recorded, sketched and photographed.

Classifying facial actions

We collected and analysed 15 hours of video footage of a wide range of naturally occurring horse behaviours from a sample of 86 horses. Known ages ranged from 4 weeks to 27 years, and the sample included horses of different breeds, coat colours, and sex. Horses were videoed using a Canon XM2 video recorder in a variety of situations, including interacting with conspecifics, humans and other animals (e.g. dogs), feeding and mating.

Each discrete facial movement identified was given a code (an Action unit - AU - or an Action Descriptor - AD) in correspondence with previous FACS systems. Where EquiFACS identified movements analogous to those in humans or other animals, the same codes have been used. In some cases, although the same muscle is used in both humans and horses the resulting action on the face is very different; in these cases the Action Unit is prefixed with an 'H' e.g. AUH13. In other cases, although the action on the face is similar in horses and humans, the underlying muscular basis differs; in these cases the AU is prefixed by a 1 (e.g. AU122). Where novel movements were noted a new Action Unit or Descriptor was created (see Table 1 and Table 2). Agreement on these classifications was reached between two trained human FACS coders (JW and BMW), a comparative anatomist (AB), and a specialist in animal behaviour (including horses) (KM).

The reliability of others to be able to learn EquiFACS and consistently code behaviour was tested with four coders, three of whom had no previous experience of FACS and one of whom had no previous experience of horses. The coders were each given the text, video examples, and practical instructions describing how to use EquiFACS effectively (see S3 Text) and asked to learn EquiFACS independently. The coders had minimum contact with the authors in this time, so that their performance would be considered a fair test of whether EquiFACS produces reliable coding when learned without training from the developers. When each coder was happy that they understood the principles of EquiFACS and felt they could reliably identify the expressions, they were asked to code 22 short video clips (containing the full range of AUs and EADs) with the manual for reference.

In accordance with previous FACS, reliability was measured using Wexler's ratio [3, 8-12, 38]. Wexler's ratio is a ratio of the number of agreements (i.e. the number of AUs on which coder 1 and coder 2 agreed x2) divided by the number of possibilities for agreement (i.e. the total number of AUs scored by both coders). This is particularly suitable for situations, such as in FACS, where there are a large number of potential codes and there is likely to be a different number of items coded by each coder. Each person's coding was compared to JW's coding, the agreement ratio was calculated for each video clip, and then an average over the video clips was taken for each coder. The overall average was 0.86 (coder 1 = 0.85; coder 2 = 0.86; coder 3 = 0.87; coder 4 = 0.85), demonstrating high reliability across the coders.

Ethical statement

This study gained ethical approval from the University of Sussex Ethical Review Committee, and was carried out in accordance with the Association of Animal Behaviour Guidelines for the Treatment of Animals in Behavioural Research. All owners gave consent for their horses to be video recorded. It should be noted that the specimen for the dissection was not euthanised for the purpose of this study, but was acquired from a veterinary centre where the horse had been euthanised for clinical reasons.

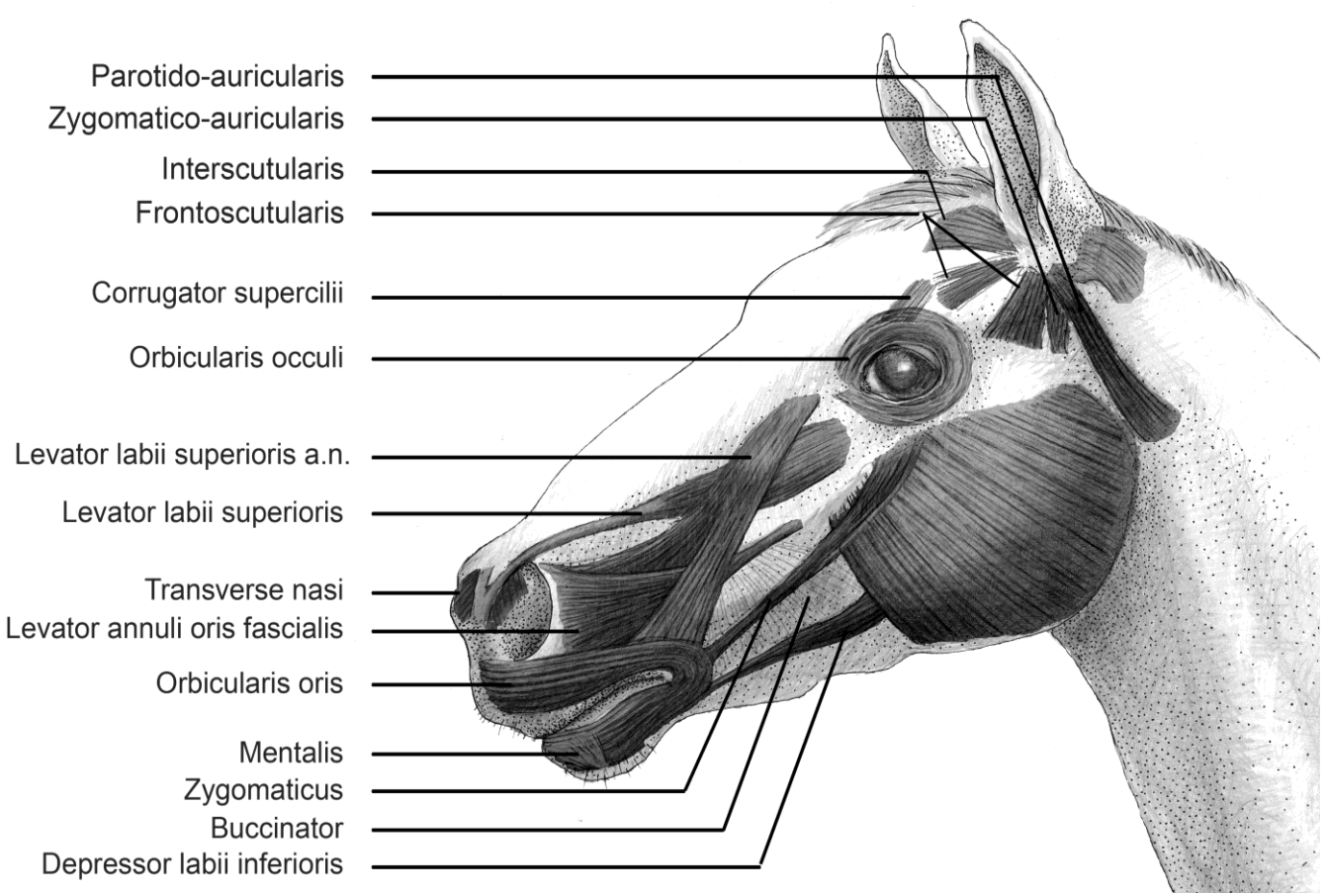


Figure 3. The facial muscles of the horse. NB. Levator labii superioris a.n. represents the levator labii superioris alaeque nasi, which is often also called the levator nasolabialis. Synonyms for the levator annuli oris fascialis muscle include the dilator nares muscle and the caninus muscle. The frontoscutularis has a frontal and a temporal arm.

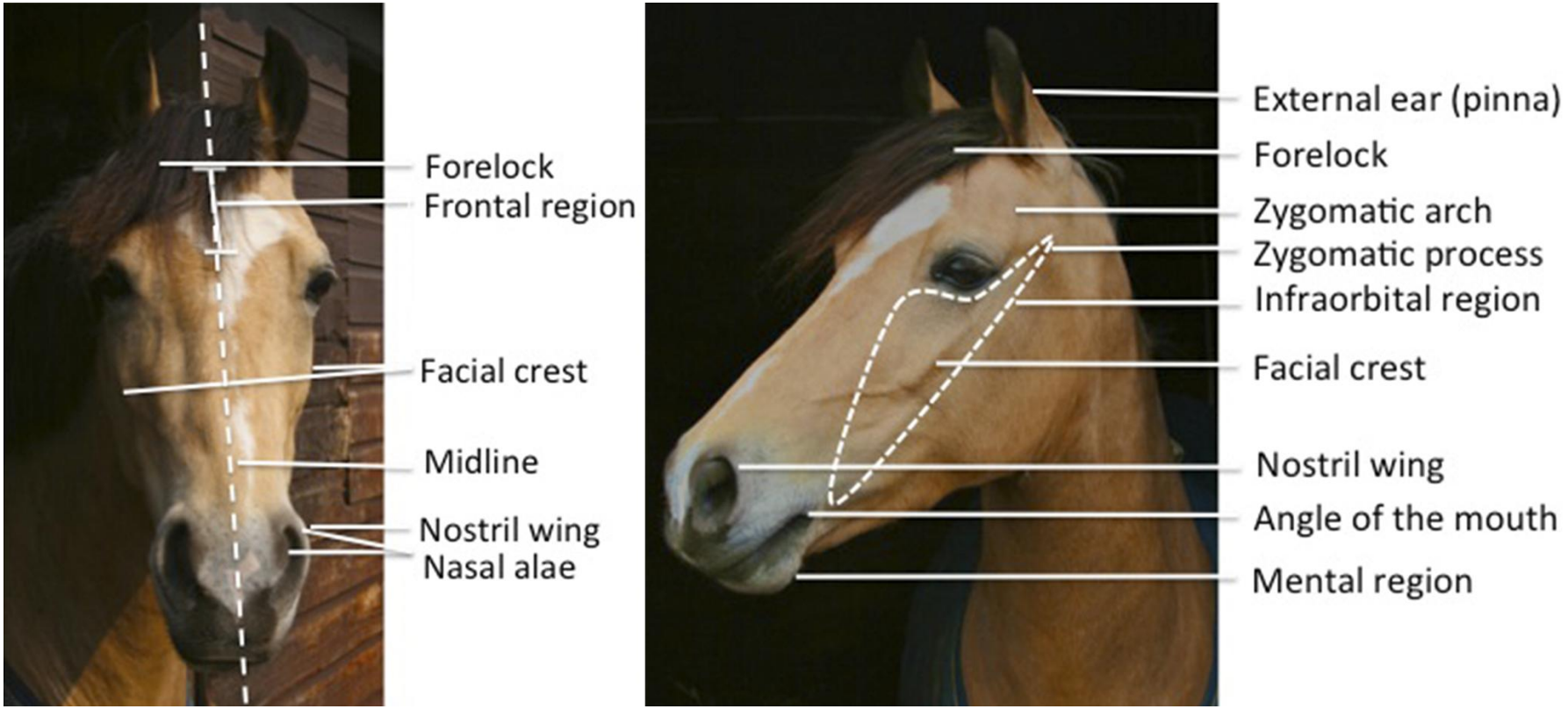


Figure 4. The facial landmarks of the horse.

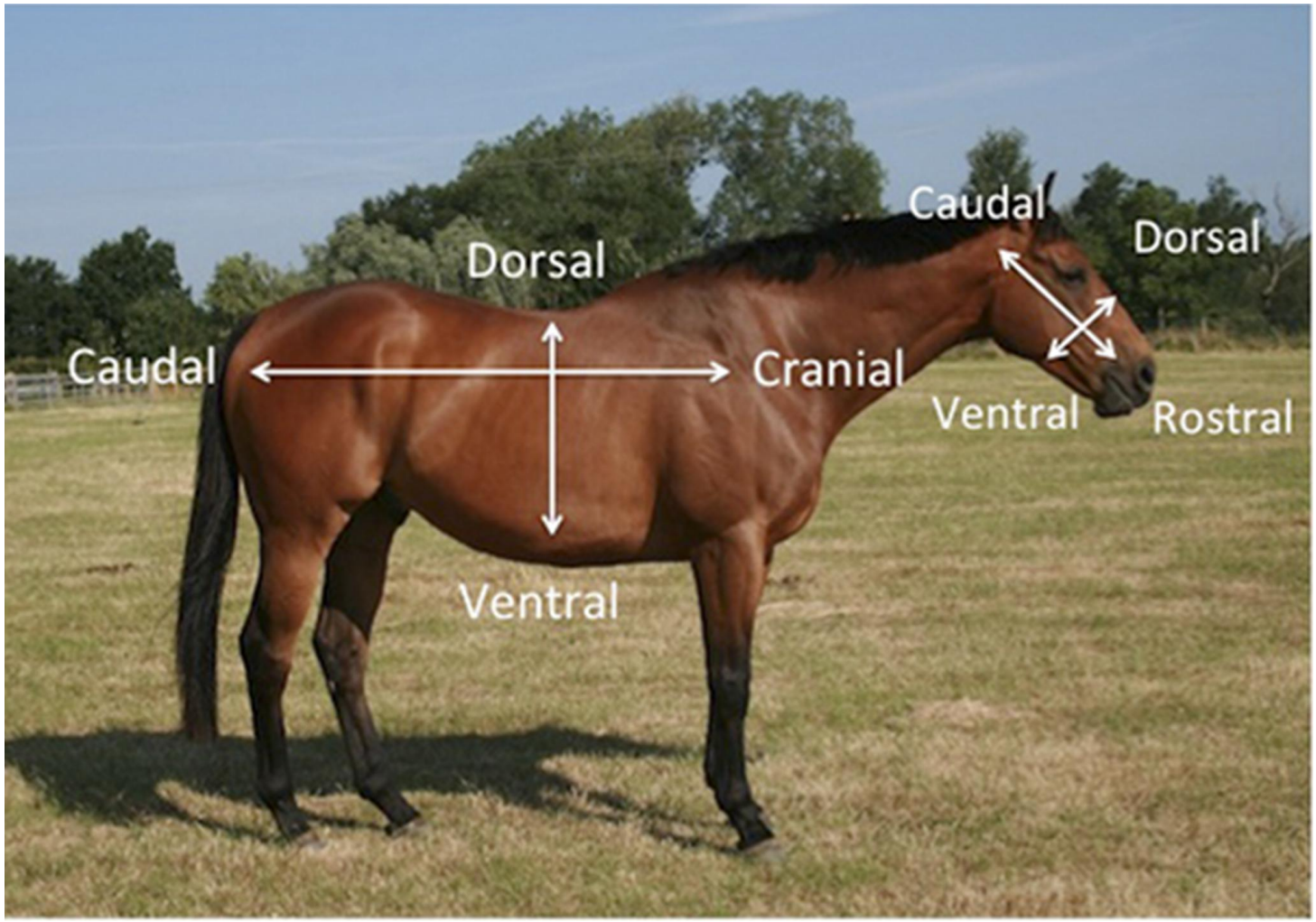


Figure 5. A guide to anatomical direction.

Results

Facial muscles of the horse

Figure 3 depicts the facial muscles of the horse (Figures 18 – 21 show the facial muscles located in our dissection). Overall, the facial muscles of horses are very complex, and although there are differences, there are a surprising number of similarities with humans and other primates. Table 1 summarises the findings in comparison to the human facial muscles. Detailed descriptions of the muscles are given with the corresponding facial actions below.

Gross observations showed that the muscles around the ear, lips, and nose of the horse were particularly large and complex, with these muscles having many attachments to the fascia, cartilage, and other muscles (Figure 19; Figure 20). In particular, many muscles of the lower face converged in the modiolus to form a large, complex muscular mass (Figure 19). This was in contrast to previous suggestions that mammals distantly related to primates, such as Perissodactyla, should have very simple facial musculature, particularly around the lips and mid-face [39, 40]. The orbital and mid-face region of the horse had numerous muscles including both a zygomatic major and minor (also termed the malaris), and an extensive obicularis occuli, although the muscles in these areas were thinner than the muscles around the ears and lower face.

There was a large amount of adipose compared to non-human primates, e.g. chimpanzee, [33]; rhesus macaques, [34] Otolemur [32] and Hylobatids [35], possibly due to the domestic situation of the horse. The adipose was particularly prevalent around the ear, but also found around the mouth (this has been cut away in the figures to allow better observation of the muscles).

Facial actions of the horse

Upper face Action Units

Action Unit 101: inner brow raiser

The anatomy of the eye area is different in horses as compared to humans, other primates, and dogs (see Figure 4 for a visual representation of the key facial landmarks of the horse and Figure 5 for a guide to anatomical direction). Horses do not have the

features, such as eyebrows or the prominent brow ridge, that are thought to accentuate brow movements in other animals. However, horses do have a facial action that raises skin above the inner corner of the eye. This is similar to the movement derived from the frontalis in humans (inner brow raiser - AU1), although in horses the action is underpinned by the levator anguli occuli medialis and the corrugator supercilii, making it analogous to the inner brow raiser seen in dogs and cats. Consequently, the code used to denote this movement in dogFACS and catFACS (AU101) is also used here.

A. Proposed muscular basis: levator anguli occuli medialis muscle, corrugator supercilii muscle (Figure 3, see S2 Text for additional comments on these muscles).

B. Appearance changes:

1. The skin above the inner corner of the eye is pulled dorsally and obliquely towards the medial frontal region (see Figure 6).
2. The skin may bulge above the inner corner of the eye.
3. Wrinkles can appear (or if already present deepen) in the area above the inner eye.
4. The shape of the skin above the eye changes: instead of following the shape of the eyelid an angular contour develops.
5. It may accompany an eye movement, although does not necessarily.
6. This action is quite subtle and can be difficult to reliably discern from a distance.

Figure 6. The area and direction of movement in AU101, inner brow raiser. The picture shows the left eye of the horse.

C. Minimum criteria to code AU101: dorsal movement of the skin above the inner eye region. See Videos 1 and 2 for examples of AU101.

Action Units 143, 145, and 47: eye closure, blink and half blink

Horses have three eyelids: upper, lower, and third (nictitating membrane). However, the third eyelid is usually only barely visible at the inner corner of the eye. The eyes of the horse are placed more laterally than the eyes of primates and dogs (40 degrees) [41], with no prominent supercilliary arch or epicanthal fold.

In horses, the orbicularis occuli muscle is mostly responsible for the actions of closing the eyes and blinking. This is in contrast to humans and other primates, who can close the eyes by relaxation of the levator palpebrae superioris alone. The eye closing action seen in horses more resembles that seen in dogs and cats, and the AUs given below correspond with the parallel actions in dogFACS and catFACS.

AUs 143 and 145 both refer to closure of the eyes, although they are mutually exclusive and cannot be coded simultaneously. The main difference between these actions is the speed at which they occur, and the extent of recruitment of the orbicularis oculi. AU143 is substantially slower than AU145. Additionally, AU145 is seen in conjunction with higher degrees of actions that resemble AU6 (the cheek raiser) in humans (e.g. raising of the infraorbital region).

However, AU6 and AU7 (lid tightener) are both omitted from EquiFACS. Both of these movements constrict the skin around the eye in primates, but are not reliably discernable as independent facial movements in horses. Horses do show some signs of AU6 within AU143 and 145 (contraction of the infraorbital region) and some contraction of the orbicularis occuli is seen that reduces the opening of the eye without closing it completely. However, these contractions are not reliably discernable as independent actions, and would be very difficult to distinguish, particularly in the field. For this reason, AU47 – the half blink, has been created, which covers all instances where the eye partially closes.

EquiFACS omits AU46, which represents a wink in humans, as AU46 refers to an intentional action. The absence of AU46 from EquiFACS is to avoid subjective decisions about whether there is intentionality of expression in horses.

Action Unit 143: eye closure

AU 143 denotes eye closure that lasts for more than half a second. This movement results from the relaxation of the levator palpebrae superioris muscle, and small actions of the orbicularis oculi.

A. Proposed muscular basis: contraction of the orbicularis oculi muscle and relaxation of the levator palpebrae superioris muscle (Figure 3, see S2 Text for additional comments on these muscles).

B. Appearance changes:

1. The upper and lower eyelids move towards each other to close the eye.
2. More surface of the upper eyelid is exposed than usual.
3. When the eyelids meet and the eye is closed, some tension might be exhibited in the skin covering and surrounding the eye.
4. The skin located above and particularly below the eye may be pulled inwards. This is most obviously seen in the infra-orbital area, which contracts superiorly (both medially and laterally).
5. Once closed the eyelids may flutter slightly (e.g. if falling asleep) and the code AU143 covers these slight movements. However, if the eyes then open fully, code subsequent movements as new movements.

C. Minimum criteria to code AU143: the eyelids must be drawn together to close the eye, and the eye must remain closed for more than half a second. See Videos 3 and 4 for examples of this action.

D. Subtle differences between AUs: AU143 is slower than AU145 (below), and the eyes close for more than half a second. If the eyes close for less than half a second score AU145. If the eyes do not close completely then do not score AU143, instead score AU47.

Action Unit 145: blink

AU145 refers to a quick eye closure that involves both the relaxation of the upper eyelid and some contraction of the lid tightening muscles. Both the timing of the action and the appearance of the eyelids, particularly the lower eyelid, can differentiate it from AU143.

A. Proposed muscular basis: contraction of the orbicularis occuli muscle and relaxation of the levator palpebrae superioris muscle (Figure 3, see S2 Text for additional comments on these muscles).

B. Appearance changes:

1. The upper and lower eyelids move towards each other to close the eye.
2. There is a rapid sequence of actions in which the upper eyelid relaxes and the lid tightening muscles contract to close the eye, followed immediately by the reversal of these actions to open the eye. There is no pause or hesitation when the eyes are closed.
3. When the eyelids meet and the eye is closed, some tension might be exhibited in the skin covering and surrounding the eye.
4. The skin located above and particularly below the eye may be pulled inwards. This is most obviously seen in the infra-orbital area, which contracts superiorly (both medially and laterally).

C. Minimum criteria to code AU145: both eyelids must move together to cover the eye, and this action must be reversed within half a second. See Videos 4 and 5 for examples of this action.

D. Subtle differences: AU145 is faster than AU143 - the eyes close for less than half a second and there is no pause while they are closed. If the eyes do not close completely then do not score AU145, instead score AU47 (half blink).

Action Unit 47: half blink

This movement has not been observed in humans, so there is no corresponding AU. Interestingly there is a corresponding movement in cats however, and the same code, AU47, is also used here.

A. Proposed muscular basis: orbicularis oculi muscle (Figure 3, see S2 Text for additional comments on these muscles).

B. Appearance changes:

1. Reduction of the eye opening by the eyelids drawing the eyelids or the skin around the eye contracting.
2. Although the opening of the eye is reduced, the eye does not close completely.
3. The skin located above and particularly below the eye may be pulled inwards. This is most obviously seen in the infra-orbital area, which contracts superiorly (both medially and laterally).

C. Minimum criteria to code AU47: a reduction in the opening of the eye. See Videos 6 and 7 for examples of this action.

D. Subtle differences: this AU differs from AU143 and AU145 because the eye never closes completely. Be cautious that the eye opening is reduced due to the movement of the eyelids towards each other, and the action is not just release of AU101, inner brow raiser (see Video 7).

Action Unit 5: upper lid raiser

AU5 pulls the upper eyelid back into the eye socket. In the usual, eyes open position, the upper eyelid covers the eye to some extent, and there is some contraction of the muscle that underlies AU5. However, AU5 denotes when the contraction goes beyond the usual, pulling the eyelid further back into the eye socket. In humans this action is very obvious, however in horses there is less of a prominent brow ridge and this action is more difficult to discern. The position of the eyelashes can be very helpful in determining the position of the eyelid.

A. Proposed muscular basis: levator palpebrae superioris muscle (Figure 3, see S2 Text for additional comments on this muscle).

B. Appearance changes:

1. Widens the eye opening.
2. Raises the upper eyelid so that it is pulled caudally and dorsally.

3. The amount of the upper portion of the eyeball exposed increases. Sclera above and around the iris may also be exposed (or increased is already present in a neutral position).
4. The shape of the upper eyelid changes as portions medially and/or laterally are pulled up.

C. Minimum criteria to code AU5: an increase in the eye opening caused by the raising of the upper eyelid. See Video 8 and 9 for examples of this action.

Action Descriptor 1: eye white increase

Horses do have white sclera around the eyes, although generally this is not visible at rest. In some situations, horses will display more of the white sclera due to a change in the opening of the eye or position of the eyeball. As this is not underpinned by a particular muscular basis, it has been labelled as an Action Descriptor, AD1. This is coded separate to AU5, as although there may be an increase in visible sclera with AU5 it is not a requirement for coding AU5. Additionally, AU5 represents widening eye opening, whereas movements of the eyeball unrelated to AU5 can also increase the visible white sclera. Consequently, AD1 and AU5 can both be scored independently, but also simultaneously.

The amount of visible white sclera is associated with the expression of fear in many animals, including humans [42, 43]. Consequently, AD1 is an essential part of EquiFACS. However, depending on the degree of interest there are two levels of coding for this action. One code simply represents an increase in the amount of white sclera exposed. The second method (which is optional and may only be useful if there is a specific interest in this expression) allows a calculation of the percentage of eye-white visible (see S4 Text).

It is necessary to establish a suitable baseline for coding these movements, as different horses will display varying amounts of white sclera at rest. It is also important to be wary of coding these movements alongside movements of the head or camera viewpoint, as these factors can also influence the amount of sclera visible

B. Appearance changes:

1. The white sclera becomes visible, or if present at rest there is an increase in the amount noticeable.
2. The white sclera may increase in any part of the eye. It is most often seen on the medial dorsal edge, but can increase around the whole circumference of the iris.
3. The eye opening may widen.
4. The eyeball may move in the socket.
5. In some horses the third eyelid (nictitating membrane) can initially appear to be white sclera in the rostral and ventral corner of the eye. If possible, obtaining a close up view should help to establish what is sclera and what is nictitating membrane.

C. Minimum criteria to code AD1: an increase in the percentage of white sclera visible. See Videos 8, 9, and 10 for examples of this action.

D. Subtle differences: the critical component of AU5 is the increased opening of the eye. Although this may reveal more visible eye white, increased white sclera can also be seen without the eye widening (in this case, code only AD1).

Lower face Action Units

The horse has an elaborate network of robust facial muscles in the lower face, with a complex innervation. This has some similarities with the facial musculature of humans, however horses also have a number of different specialisations (Table 1). The movements of the lower face are complicated and often many actions are seen together in a rapid, fluid sequence.

Action Unit 10: upper lip raiser

A. Proposed muscular basis: levator labii superioris alaeque nasi muscle (also called levator nasolabialis), transverse nasi muscle (Figure 3, see S2 Text for additional comments on these muscles).

B. Appearance changes:

1. The centre of the upper lip is raised straight up, and in strong actions the rest of

the upper lip may also be partially raised (see Figure 7).

2. The upper teeth, and in strong actions the upper gum, will become visible.
3. The upper lip does not evert as it does in the lip curl (AU 122) or protrude away from the lip as in the lip pucker AU18, (unless AU10 is combined with these movements). Instead, the upper lip edge may appear to roll inwards.
4. Transverse wrinkles are seen across the front of the nose, and these may extend up the face (except in very mild actions). These wrinkles are most obvious from a frontal view, but in strong actions the corrugation of the skin can also be seen in profile.
5. Wrinkles may also appear behind the caudal edge of the nostril wing.
6. Although the wrinkles may extend up the nose, and there may be some tension in the skin of the midface, AU10 does not affect the infraorbital region or the skin around the eyes.
7. The nostril wings may widen and rise. However, this is only due to the movement of the upper lip displacing the skin in the nostril area, rather than any movement of the nostril wing itself.

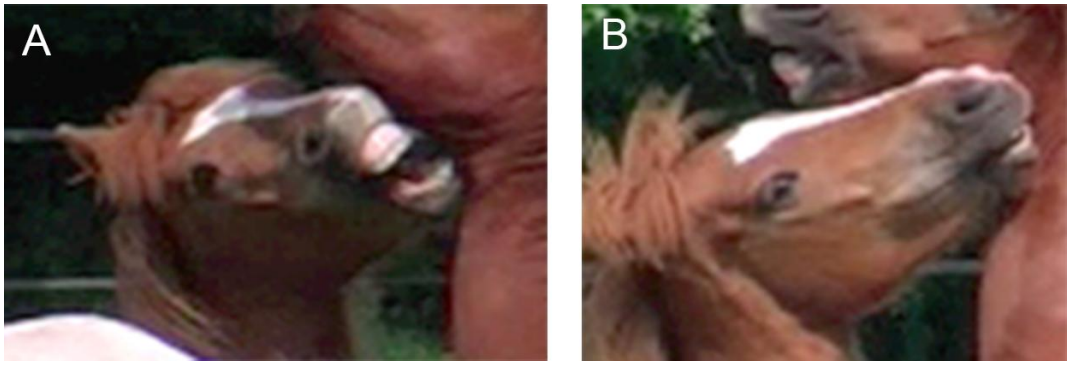


Figure 7. AU10, upper lip raiser (+ 16 + 17 + 25 + 27 + AD1 [AD1 seen in part B only]). Part A shows appearance changes 1,2,3, 4, and 7; part B shows appearance changes 2, 4, 5, 6, and 7.

C. Minimum criteria to code AU10: the central part of the upper lip is raised. See Figure 7 and Videos 11 and 12 for examples of this action.

D. Subtle differences: similar transverse wrinkles occur across the bridge of the nose in both AU10 and AU122, upper lip curl. Additionally, both actions raise the upper lip exposing the teeth and gums. However, the lip does not evert in AU10, whereas the lip

curls up and round in AU122. If the inside of the upper lip (rather than just the gum) is visible, code AU122 instead of AU10. Movement of the nostril wings with AU10 are in conjunction with the movement of the upper lip. This is unlike the movement of the nostril seen in AUH13, nostril lift, which is isolated to the nostril wing. It is difficult to code AU10 and AUH13 together unless a very strong action of AUH13 has occurred or the actions occur sequentially.

Action Unit 12: lip corner puller

In primates, dogs, and cats, the zygomatic major muscle (often termed zygomaticus muscle in dogs and cats) muscle pulls the lip corners obliquely towards the cheekbone. Horses also have a zygomatic major muscle (generally termed the zygomaticus muscle) that pulls the lip corners back. However, this action has a slightly different appearance in horses due to their different facial morphology.

A. Proposed muscular basis: zygomatic major muscle (also termed zygomaticus muscle – Figure 3, see S2 Text for additional comments on this muscle).

B. Appearance changes:

1. The lip corners are pulled back caudally.
2. This may expose the teeth and gums.
3. In strong actions, the medial section of the lips may also be drawn back exposing the teeth and gums. However, this is due to the stretching of the skin of the lips, rather than a genuine movement up or down. Consequently, even when the middle of the lips are drawn back by AU12, wrinkles on the nose or chin boss are not seen. If these signs are evident consider coding AU10, upper lip raise, or AU16, lower lip depressor, in addition.
4. The mouth is often open or repeatedly opening and closing with this movement.
5. The lip corners may pouch and wrinkle.
6. The lips are elongated.
7. The action of AU12 is not seen on the rest of the face, as it is in primates. There is no raising of the infraorbital region.

C. Minimum requirement to code AU12: the corners of the lips must be pulled towards the ears. See Videos 13, 14, and 15 for examples of this action.

D. Subtle differences: AU12 is likely to be confused with (or act with) AU113 – see section on subtle differences in AU113.

Action Unit 113: sharp lip puller

This action resembles AU13, sharp lip puller, in humans, where the corners of the lips are pulled sharply up towards the upper jawbone. However, in horses a different muscle to that used in humans causes this movement. To account for this differing muscular basis, this action has been given the code 113.

A. Proposed muscular basis: levator labii superioris alaeque nasi muscle (also known as the levator nasolabialis, see Figure 3 and S2 Text for additional comments on this muscle).

Figure 8. The location and direction of movement in AU113, sharp lip puller (indicated by the arrow). The lips are in the neutral position. AU113 is very difficult to code from photographs unless there is a very strong action, or it is combined with AU12, lip corner puller.

B. Appearance changes:

1. The corner of the upper lip is pulled up towards the bridge of the nose (Figure 8).
2. The medial section of the upper lip does not rise (unlike AU10, upper lip raiser; see section on subtle differences below). This means that AU113 is only

discernable from a profile view, as there is little or no observable action from a frontal perspective.

3. The skin above the upper lip, extending caudally up the face, also raises and wrinkles may develop in this area.
4. Wrinkles running along the face may appear under the nostril wing.
5. There may be some bulging under the skin in the area where the levator labii superioris alaeque nasi lies.
6. This can be a very discrete movement, although is generally more clear if the video clip is watched in slow motion.
7. Some tension may be exerted on the lower lip and the skin around the mouth. However, this tension is only minimal and in the direction of the upper lip movement. If there is movement of the lower lip in other directions, then consider coding additional AUs.

C. Minimum criteria to code AU113: oblique (caudal and dorsal) movement of the lateral part of the upper lip. See Videos 14, 16, and 17 for examples of this action.

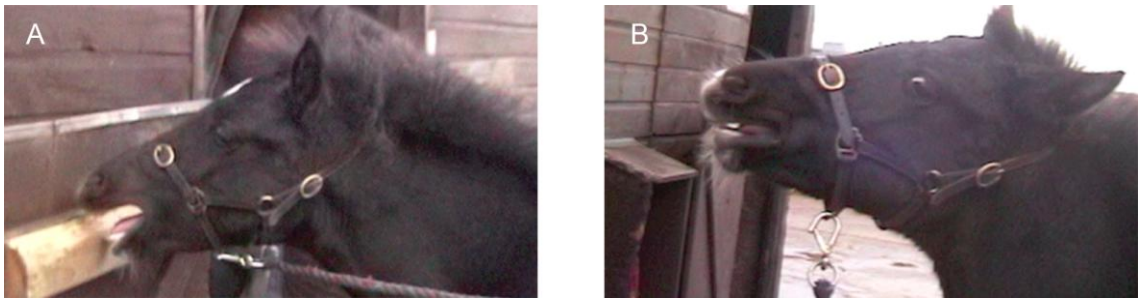


Figure 9. AU12, lip corner puller, without AU113, sharp lip puller, (A) and AU12 with AU113 (B). Note the difference in the shape at the corner of the mouth. AU12 produces a curvature at the mouth corner; however, when AU113 is applied with AU12 the mouth has an angular appearance at the top corner, with corresponding wrinkles in the skin surrounding the mouth. See Video 15 for a demonstration of these actions acting simultaneously.

D. Subtle differences: AU113 differs from AU12 in the direction of the movement. In AU12 the skin is drawn back at the corners of the mouth, whereas in AU113 the skin is drawn up towards the bridge of the nose. AU113 and AU12 do sometimes occur

together, but when this happens the actions occur sequentially with AU113 generally occurring first, and so the timing of the actions can help distinguish them. Additionally, when AU113 is acting with AU12 the curve at the corner of the mouth becomes much more angular (Figure 9). AU113 also differs from AU10, because the movement is restricted to the corners of the lips so the medial section of the upper lip does not rise.

Action Unit H13: nostril lift

In humans the levator annuli oris fascialis muscle (also known as the caninus muscle or dilator naris lateralis muscle) acts on the upper lip and is responsible for AU13. However, in horses the levator annuli oris fascialis has no action on the upper lip. Instead, it draws back the caudal wing of the nostril. Consequently, although this movement has the same muscular basis in horses and humans, it is labeled AUH13 in EquiFACS to highlight the difference in action and appearance.

A. Proposed muscular basis: levator annuli oris fascialis muscle (also known as the caninus muscle or dilator naris lateralis muscle, see Figure 3, see S2 Text for additional comments on this muscle).

B. Appearance changes:

1. The caudal (back) edge of the nostril is pulled up and drawn round laterally (Figure 10).
2. This changes the shape of the nostril at this edge, from a smooth curve to a more angular shape.
3. The nostril is elongated.
4. AUH13 can cause the skin behind the nostril to wrinkle, or cause wrinkles to deepen if already present. This appearance change is particularly useful for determining weaker actions of AUH13.
5. Some horses may hold this movement on the face for a long time, and it might become confused with the neutral shape of the nostril. As there is no movement that can lower the caudal edge of the nostril, a drop in the nostril wing indicates that AUH13 was present.
6. This movement can be unilateral and often is seen performed on only one side of the face. For this reason, unless you can see both sides of the face and are certain that a bilateral movement has occurred code a unilateral action of AUH13.

7. As this movement produces a very distinctive shape to the nostril, it is possible to code this from photographs when it has been applied at high intensities.

C. Minimum criteria to code AUH13: the caudal edge of the nostril must move laterally and up, or if already present on the face the caudal edge of the nostril must drop down as the levator annuli oris fascialis muscle relaxes. See Figure 10 and Videos 18 and 19 for examples of this action.

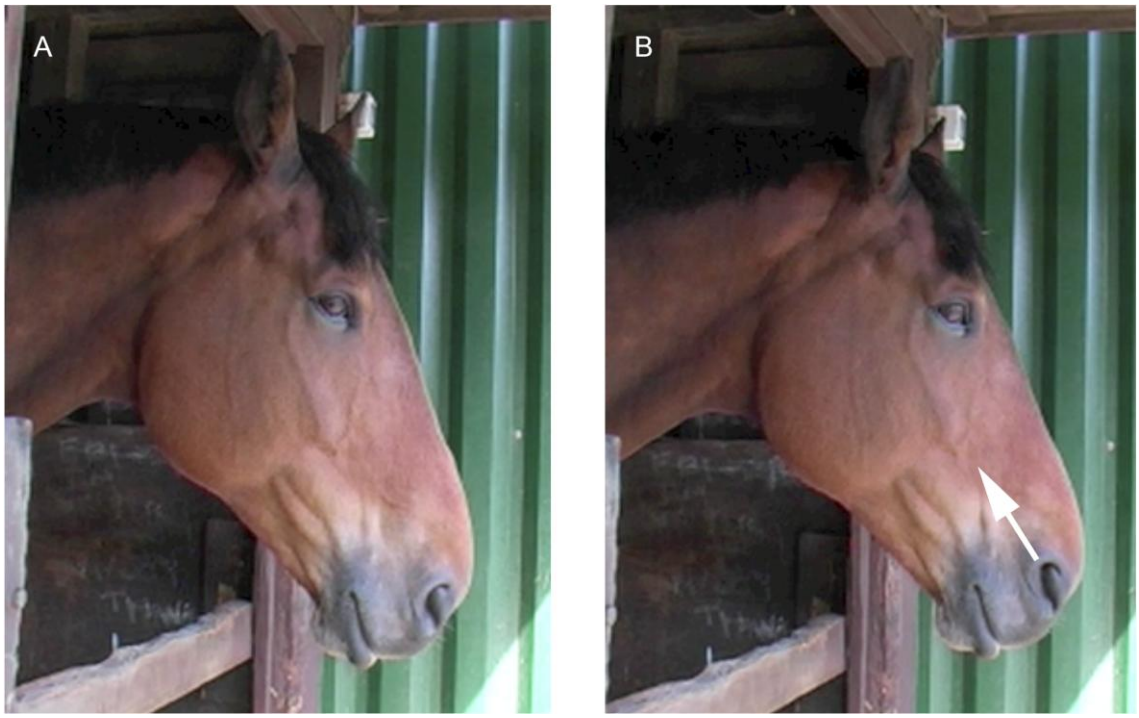


Figure 10. Direction and area of AUH13, nostril lift. The picture on the left shows the neutral face, the picture on the right shows the face with a mild action of AUH13 applied. The arrow illustrates the location and direction of movement.

D. Subtle differences: AUH13 is most likely to be confused with AD38, nostril dilator. One key difference is that AUH13 will not act on the rostral (front) edge of the nostril, whereas AD38 will. If there is some action on the rostral edge of the nostril, consider coding AD38 instead of (or, if sequential actions are seen, in addition to) AUH13. Additionally, in AUH13 there will be definite caudal movement of the skin behind the nostril wing, which is not seen in AD38.

Action Unit 16: lower lip depressor

AU16 refers to the action of the lower lip being pulled down by the depressor labii inferioris muscle, as it does in primates. It must be visibly pulled down, rather than just relaxed. If the lower lip is hanging down due to relaxation, then code AD160, lower lip relax. The images, video, and descriptions deal with AUs 16+25, lips part. Usually, when there is an action of 16 the lips part and are scored as 16+25, 16+25+26, jaw drop, or 16+25+27, mouth stretch.

A. Proposed muscular basis: depressor labii inferioris muscle (Figure 3, see S2 Text for additional comments on this muscle).

B. Appearance changes:

1. The lower lip is pulled down ventrally.
2. The lower lip is stretched and pulled down laterally.
3. The skin covering the mental region can be stretched laterally and down, flattening and occasionally wrinkling the skin in this area.
4. In stronger movements this will expose some of the teeth and gums.
5. The pink flesh on the inside of the lower lip may show.
6. May cause the lower lip to protrude or flatten (depending on the individual).
7. It is often seen in combination with other AUs, and when seen with AU17 the combination produces a distinct shape – see the sections on subtle differences.
8. Often 16 may present more strongly on one side of the face than the other. However, only code a unilateral action if signs of 16 are completely absent from one side of the face.

C. Minimum criteria to code AU16: the lower lip must be pulled down. See Videos 20 and 21 for examples of this action.

D. Subtle differences: AU16 is a definite movement, whereas when the lip is lowered by AD160, lower lip relax, it is due to relaxation of the muscles. AU16 is often combined with AU17, chin raiser, which changes the appearance of the lower lip – see the details of combination AU16 + 17 and Video 21. Finally, when the mouth is open or the skin around the mouth is stretched (e.g. in AU12, lip corner puller) then the skin of the lower lip will be stretched and some of the lower teeth or gum might show. To code

16 in these situations try and establish whether the lowering of the lip is greater than would be expected by skin stretching or mouth opening alone.

Action Descriptor 160: lower lip relax

As in chimpanzees, this action occurs from the relaxation of the lower lip and the lip is pulled down by its weight alone. See Video 22 for an example of this action.

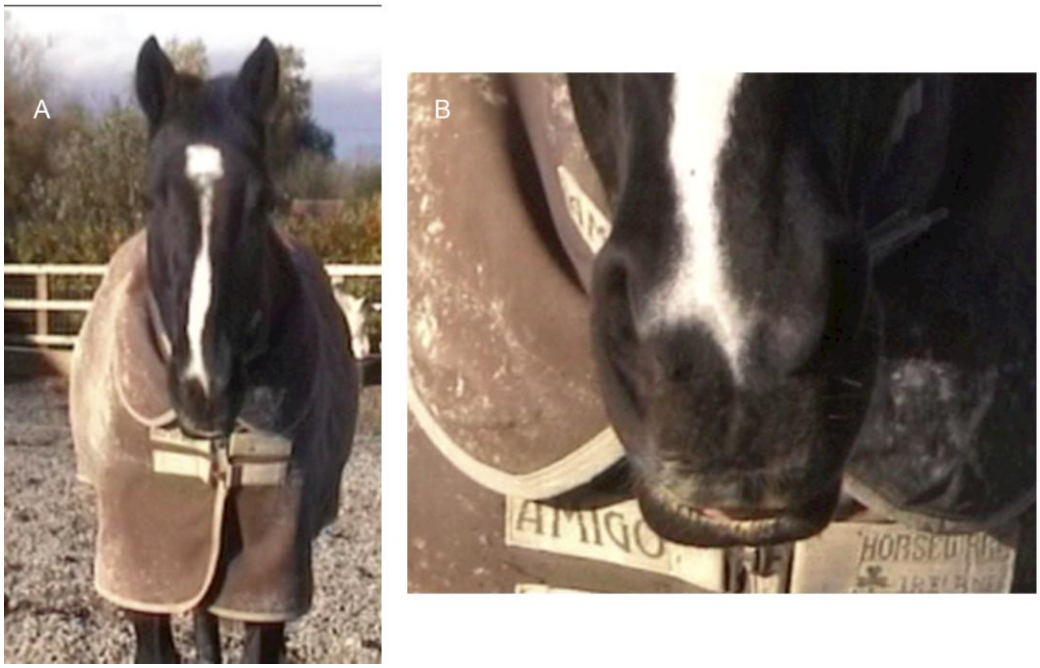


Figure 11. AD160, lower lip relax, seen from a difference and close up. Note that from a difference, although subtle, AD160 can be identified by the visible pink flesh from the inside of the lower lip.

B. Appearance changes:

1. The lower lip is visibly relaxed and hangs loose with no tension.
2. Often this will cause the lips to part and some of the teeth and gums may become visible.
3. Similarly, the pink flesh on the inside of the lower lip may show (see Figure 11).
4. The outline of the lower lip will change; there will be less definition in the lip and the lower lip will not meet the upper lip (see Figure 12). Establishing a neutral position for the lower lip can help in cases where AD160 is slight.
5. As this action is a relaxation of the face, rather than the application of a specific muscle, the onset of this action can be gradual. However, often the release of this action (when the lower lip tightens to resume the neutral position) is much

more defined. Watching for the release of AD160 can help to establish whether it was present or not.

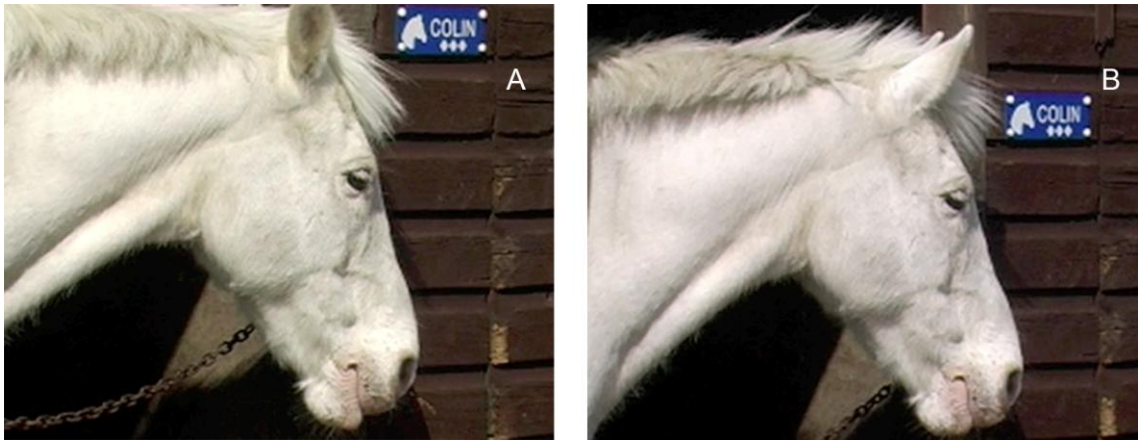


Figure 12. Profile view of AD160, lower lip relax. Panel A demonstrates the outline of the lower lip when AD160 is present, and panel B when AD160 is released.

Action Unit 17: chin raiser

AU17 is underpinned by contraction of the mentalis muscle, which arises in the prominence of the mental region (also known as the chin) and inserts into the mental region. Consequently AU17 tightens the skin in the mental region and pushes the skin up towards the lower lip and nose.

A. Proposed muscular basis: mentalis muscle (Figure 3, see S2 Text for additional comments on this muscle).

B. Appearance changes due to AU17:

1. The skin covering the mental region tightens and moves up towards the upper lip. This alters the outline of the lower lip, making the mental region more defined.
2. The skin covering the mental region and lower lip are pushed upwards.
3. Wrinkles may appear in the mental region as the skin is stretched and tightened.
4. The lower lip may protrude, particularly in strong actions. This may cause a prominent ridge under the lower lip.

5. This action of the lower lip may also push the upper lip up slightly.

C: Minimum criteria to code AU17: the skin covering the mental region and lower lip must be pushed up. See Videos 23 and 24 for examples of this action.

D. Subtle differences: see sections following AU16 and combination AU16 + 17.

Action Unit combination 16 and 17

In combination AU16 + 17 the action of the depressor labii inferioris muscle (pulling down the lip) is mediated by the action of the mentalis muscle (pushing up the skin of the mental region). This causes a distinct appearance, where the lip can still be pulled down, but to a lesser extent than when AU16 is acting alone.

B. Appearance changes due to AU16 and AU17:

1. The skin tightening action of 17 counteracts some of the lowering action on 16. This means that in combination 16 + 17, generally only the front section of the lower lip is pulled down, unlike in 16 alone where the whole lower lip may be pulled down and away from the jaw (see Figure 13).
2. This combined action of the muscles underpinning 16 and 17 may cause the lower lip to protrude as the skin over the chin is held tight while the lip is pulled down. The shape of the lower lip may be more square than when 16 is applied in isolation, due to the tightening action of 17 (see Figure 13).
3. At lower intensities the location of the movement can help to distinguish between AU16 and the combination of AU16 + 17. If the lip lowering seems to be coming from the insertion of the depressor labii inferioris then AU16 is acting alone, but if the lip lowering action seems to be only in the very front of the lip then 17 is acting with 16. For this reason it is much easier to distinguish between these actions from a ¾ or profile view. It can be difficult and sometimes impossible to distinguish between 16 alone and combination 16 + 17 from a frontal view.
4. When these action units are combined with very strong actions of AU27 be aware that the skin covering the chin will be stretched due to the jaw stretching action of 27, and this can interfere with the appearance changes of AU16 and/or

17. In these cases rely on the other appearance changes to help you establish whether AUs 16 and or 17 have occurred.

C. Minimum criteria to code combination AU16 + 17: both a lowering of the lower lip and a raising of the skin covering the mental region. See Figure 13 and Videos 25 and 26 for examples of this action.

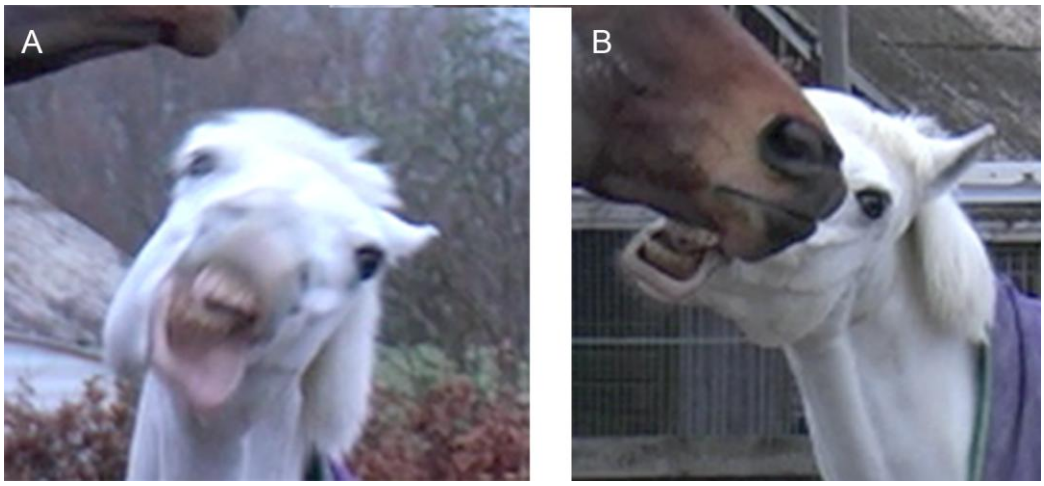


Figure 13. The lower lip when AU16, lower lip depressor, is acting in isolation (A) and in combination with AU17, chin raiser (B). Note the square shape of the lip and definition in the skin covering the mental region in combination AU16+17.

Action Unit 18: lip pucker

AU 18 draws the lips medially, causing the lips to pucker and protrude. In humans this is generally seen in both lips, however in horses this action is mostly seen in the top lip only.

A. Proposed muscular basis: orbicularis oris muscle, incisivii labii muscle (Figure 3, see S2 Text for additional comments on these muscles).

B. Appearance changes:

1. Pushes the lip forward and draws the lip medially, as if a drawstring were being pulled around the top lip.
2. Shortens the mouth from a frontal view, making the mouth opening smaller and

rounder, and the lip appear tight.

3. Elongates the lip from a profile view, drawing the top lip rostrally.
4. The lip becomes tense and moves away from the teeth. It may look as though it is hanging over the bottom lip.
5. Horizontal wrinkles may appear along the face.
6. There is no eversion of the lip in AU18. If this is seen then AU122, upper lip curl, or 18+122 must be scored.
7. Very strong actions of AU18 may part the lips and draw the top lip up slightly.
8. Often the lip can be drawn round to one side in this movement. This is due to AU18 acting more strongly on one side of the lip that the other. Do not score this as a unilateral action unless there is no trace of 18 on one side of the face.

C. Minimum criteria to code AU18: the top lip is tightened and drawn medially and forward away from the teeth. See Videos 27, 28, and 29 for examples of this action.

D. Subtle differences: the strands of muscle that underpin AU18 are also recruited in AU122, which leads to some shared appearance changes. The critical criterion to distinguish between these actions is whether there is any eversion of the top lip. If not, then score AU18; however, if there is score AU122. Also look for whether the action is isolated to the top lip (AU18), or whether it spreads further up the nose (AU122). AU18 and 122 can only be scored together if their actions are seen sequentially.

Action Unit 122: upper lip curl

AU122 resembles AU22, the Lip Funneler, which is seen in humans and chimpanzees. However, in horses this movement has a partially different muscular basis and involves the transverse nasi and levator labii superioris muscles in addition to the orbicularis oris muscle. In AU122 the lip is pushed forward and curls/flares. By definition this must part the lips, and so must always be scored with an AU25, lips part, and this action is usually only seen in the top lip.

A. Proposed muscular basis: levator labii superioris muscle, transverse nasi labii muscle, orbicularis oris muscle (Figure 3, see S2 Text for additional comments on these muscles).

B. Appearance changes:

1. The upper lip everts and curls up.
2. The action is focused on the medial portion of the upper lip.
3. The upper lip will pucker, sharing some of the appearance changes with AU18 – see the section on subtle differences for help distinguishing the two actions.
4. There is distinctive wrinkling across the nose, and along the side on the nose (see Figure 14). This wrinkling is also seen in AU10, upper lip raiser, see the section on subtle differences for help distinguishing the two actions.
5. This action may be held for a long time.

Figure 14. An example of the wrinkles seen with AU122, upper lip curl. These wrinkles are also characteristic of AU10, upper lip raiser, however in AU10 there is no puckering or eversion of the lip.

C. Minimum criteria to code AU122: the lips must part and the upper lip must curl up. See Videos 30, 31, and 32 for examples of this action.

D. Subtle differences: AUs122 and 10, upper lip raiser, are difficult to distinguish at low intensities. Look at whether the upper lip puckers and moves away from the gum; if so code AU122, but if not then code AU10. At higher intensities the upper lip curl/eversion is important to distinguish AU122 from AU10, in which the upper lip only lifts and does not curl. Similarly, weak actions of AUs122 and 18, lip pucker, are difficult to discriminate, as both involve lip puckering. Watch for the pulling of the lip from higher up the nose, in between the nostrils, and transverse wrinkles across the nose that only occur in AU122 and not 18. If the upper lip is puckered but not curled up then code AU18. Be aware that these actions may often occur in rapid succession (e.g.

Video 32).

Action Unit 24: lip presser

A. Proposed muscular basis: orbicularis oris muscle (Figure 3, see S2 Text for additional comments on this muscle).

B. Appearance changes:

1. Presses the lips together, without pushing up the skin covering the mental region (if this is seen also code AU17, chin raiser).
2. Lowers the upper lip and raises the lower lip to a small extent.
3. Tightens and narrows the lips.
4. May cause a bulging of the skin above the upper and/or lower lip.
5. Both lips are drawn in towards the mouth.

C. Minimum criteria to code AU24: the lips must be pulled in and pressed together. See Video 33 and 34 for examples of this action.

D. Subtle differences: the pressing together in AU24 is caused by a muscular movement, and not just the movement of the jaws, e.g. in chewing. If lower lip alone is moved up (and this pushes up the top lip too) then code AU17. AU24 and 17 can occur together, in this case look for action further down the lower lip, in the skin covering the mental region (Video 34).

Action Units 25, 26, and 27: lips part, jaw drop, and mouth stretch

These three AUs describe mouth opening, including separation of the lips and the teeth. Although there could be an argument for classifying these as Action Descriptors rather than Action Units, we have described them as Action Units here to remain consistent with previous FACS. AU25 denotes separation of the lips, so can be coded independently or with AU26 or 27. AU26 and 27 both refer to parting of the jaw; and so can be coded with or without 25. However, AU26 and 27 are mutually exclusive as they designate different types of jaw opening, and so AU26 and 27 must not both be coded for the same movement. Although AU26 and 27 both code opening of the mouth, they refer to different actions. AU26 refers to the dropping of the lower jaw that is caused by relaxation of muscles. However, AU27 refers to a more purposeful movement where the

jaw is stretched apart by contraction of muscles.

Action Unit 25: lips part

A. Proposed muscular basis: orbicularis oris, levator labii superioris, levator nasolabialis, depressor labii, transverse nasi, zygomaticus muscles, or the action may not be muscular but may be caused by opening the jaw (Figure 3, see S2 Text for additional comments on this muscle).

B. Appearance changes:

1. The lips are separated at any point around the mouth.
2. There is a gap seen between the top lip and bottom lip.
3. In stronger actions the gums or teeth may be visible.

C. Minimum criteria to code AU25: the lips must be seen to part at some point. See Video 35 for an example of this action.

Action Unit 26: jaw drop

A. Proposed muscular basis: this movement is not caused by the facial muscles, but by muscles such as the masseter.

B. Appearance changes:

1. The lower jaw is lowered and teeth separation can be clearly seen or at least inferred.
2. The movement is relaxed and the jaw does not open so much that it is causing an obvious stretch.
3. Although AU26 can be coded with AU25 it does not have to be. If there are signs of jaw lowering (i.e. you can see the skin move) without the lips parting, then score AU26 alone.

C. Minimum criteria to code AU26: there must be movement of lower jaw either seen through movement of the overlying skin, or teeth separation. See Video 36 for an example of this action.

Action Unit 27: mouth stretch

A. Proposed muscular basis: this movement is not caused by the facial muscles, but by muscles such as the masseter.

B. Appearance changes:

1. The lower jaw is pulled down and teeth separation can be clearly seen.
2. The movement is purposeful and the mouth appears to be clearly stretched open, rather than the more relaxed jaw lowering in AU26.
3. It is likely that AU27 will be coded with AU25, as the degree of jaw opening in AU27 is usually strong enough to part the lips.
4. The lips may retract or change shape to accommodate the degree of mouth opening.

C. Minimum criteria to code AU27: the jaw must be parted and this action must be through an obvious mouth stretch. See Video 37 for an example of this action.

Ear Action Descriptors

Ear actions do not feature in many primate FACS, as the auricular (ear) muscles of many the primates are gracile and vestigial. Rhesus macaques are an exception where ear action descriptors have been described [8, 10], as they are for the domestic dog [12] and cat [13].

Horses have an extremely complex and robust set of auricular muscles that allow a great range and specificity of movement. However, the ears of horses are obvious and large, with little variation in the shape of the pinna (external ear), making it relatively easy to code ear movements. Often both of the ears are visible simultaneously, unlike the other movements described in EquiFACS where one side of the face is likely to be obscured. If one ear is out of sight it cannot be assumed that any action seen in the visible ear is bilateral. The ears can, and often do, move independently, and so it should be carefully noted if a movement is unilateral or if one ear is out of sight (AD 75).

Action descriptors are given rather than specific action units, as due to the complex muscular network around the auricle it is impossible to know the exact basis of the ear movements. For this reason section **A. Proposed Muscular Basis,** is omitted from the

descriptions below. Before coding ear movements in horses, a neutral position for each individual must be determined. This helps to distinguish between genuine ear movements and a release of a previous movement that is returning the ears to neutral. The ideal time to obtain sight of a neutral position is when the horse is resting (e.g. Figure 15).

Figure 15. The general neutral ear position of the horse, although there is some individual variation.

Ear Action Descriptor 101: ears forward

B. Appearance changes:

1. The ear(s) are turned or swivel forward (rostrally).
2. From a frontal view the amount of inner ear seen increases.
3. From a profile view the amount of inner ear seen may decrease (although this depends on the strength of the movement).
4. The distance between the tips of the pinna decreases.

C. Minimum criteria to code EAD101: a rostral rotation of the pinna. See Videos 38 and 39 for examples of this action.

Ear Action Descriptor 102: ear adductor

B. Appearance changes:

1. The ear(s) are pulled towards the midline (i.e. adducted).
2. The distance between ears decreases, this is particularly obvious in the tips of the pinna.
3. If viewed in profile, then the amount of inner ear/ear opening visible will

increase.

4. This is a separate movement to EAD101 (although EAD101 and EAD102 may be coded together).

C. Minimum criteria to code EAD102: movement of at least one ear towards the midline. See Video 40 for an example of this action.

D. Subtle differences: in EAD101 the ears may become closer together as the ears are brought round and forward in a rotational movement. However, unless the movement clearly pulls the ears in towards the midline, do not code EAD102. Only use EAD102 when there is a movement in towards the midline that draws the medial side of the pinna to a more acute angle on the head, and when this movement cannot be explained by a rotational movement alone. Often when EAD101 and EAD102 are present together the sequential application of these actions is visible.

Ear Action Descriptor 103: ear flattener

B. Appearance changes:

1. The ear(s) are flattened and abducted (Figure 16).
2. From a profile view the amount of inner ear visible will decrease and the angle of the ears relative to the midline will increase.
3. This is often (although not always) seen with EAD104, ear rotator. In this case the combined action of EAD103+EAD104 alters the appearance of the flattening movement compared to when EAD103 is acting alone.
4. When applied with EAD104 the angle of the ears to the front of the nose will increase (Figure 16) and in a frontal profile, the ear(s) may disappear from view behind the head (Figure 17).

C. Minimum criteria to code EAD103: the ears are pulled caudally. See Videos 41 and 42 for examples of this action.

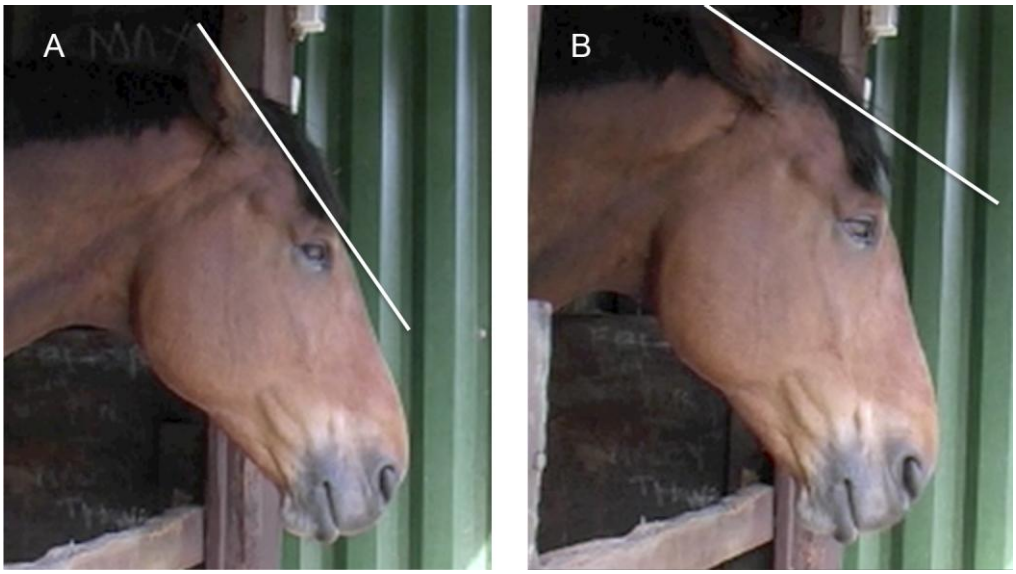


Figure 16. The starting ear position (A) and then with EAD103, ear flattener, applied (B). Note how the angle of the ears to the front of the face alters.

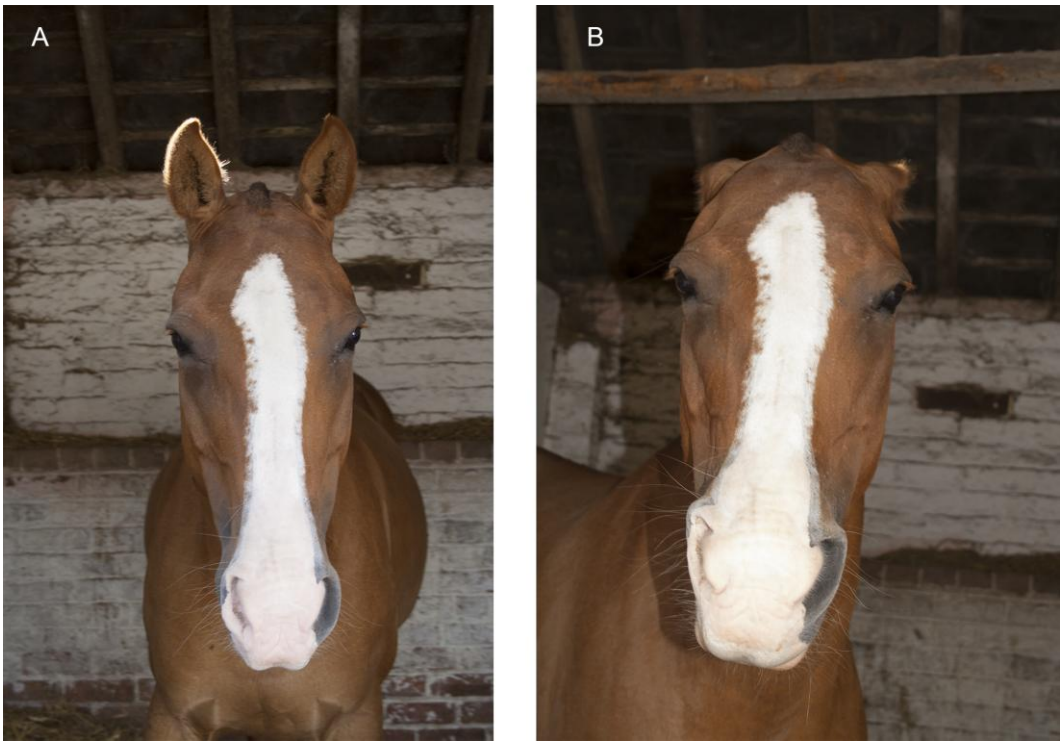


Figure 17. The starting ear position (A) and then with EAD 103, ear flattener, and EAD104, ear rotator, applied. An example of how the ears can almost disappear in a frontal view of a strong action.

Ear Action Descriptor 104: ear rotator

B. Appearance changes:

1. The ears are rotated laterally and dorsal/caudally. The opening of the inner ear is turned outwards.
2. This is the opposing movement to EAD101, ears forward, and the ears will swivel in the opposite direction.
3. Ensure that this is not confused with EAD103, ear flattener. EAD104 is a rotational movement, whereas EAD103 flattens the ears to the head/neck. The two actions may happen simultaneously.

C. Minimum criteria to code EAD104: the pinna rotates caudally. See Video 43 for an example of this action.

Miscellaneous actions and supplementary codes

A variety of miscellaneous actions and supplementary codes are given in S5 Text. Whilst these do not describe facial movements, they denote actions that can influence the coding of facial expression.

Table 1. Summary of Action Units in EquiFACS compared to Human FACS.

Action Unit	**Muscles**	**In Human FACS**
101 Inner brow raiser	Levator anguli occuli medialis	Resembles AU1, which is underpinned by the frontalis. The frontalis is present in horses (interscutularis) but does not seem to raise the brow region.
143 Eye closure and 145 Blink	Orbicularis occuli, Levator palpebrae superioris	Resembles AU43 and 45, but these are underpinned by the levator palpebrae superioris alone.
47 Half blink	Orbicularis occuli	Not described
5 Upper lid raiser	Levator palpebrae superioris	Same code and muscles
10 Upper lip raiser	Levator labii superioris, transverse nasi	Same code and muscles
12 Lip corner puller	Zygomatic major	Same code and muscles
113 Sharp lip puller	Levator labii superioris alaeque nasi	Not described, but similar visual properties to AU13.
H13 Nostril lift	Levator annuli oris fascialis	Same muscles as AU13, although different action and visual appearance
16 Lower lip depressor	Depressor labii inferioris	Same code and muscle
17 Chin raiser	Mentalis	Same code and muscle
18 Lip pucker	Orbicularis oris, incisvii labii	Same code and muscles, although more likely to occur in both lips in humans
122 Upper lip curl	Levator labii superioris; transverse nasi	Similar action to AU22, but more likely to occur in both lips in humans, and has a different muscular basis.
24 Lip presser	Orbicularis oris	Same code and muscle
25 Lips part	Depressor labii, or relaxation of the mentalis or orbicularis oris	Same code and muscles
26 Jaw drop	Masetter, temporal and internal pterygoid relaxed	Same code and muscles
27 Mouth Stretch	Pterygoids, digastric	Same code and muscles

Table 2. Summary of Action Descriptors in EquiFACS compared to Human FACS.

Action Descriptor	**In Human FACS**
1 Eye white increase	Not described
101 Ears forward	Not described
102 Ear adductor	Not described
103 Ear flattener	Not described
104 Ear rotator	Not described
160 Lower lip relax	Not described
19 Tongue show	Same code
29 Jaw thrust	Same code
30 Jaw sideways	Same code
133 Blow	Similar to AD33, blow
38 Nostril dilator	Same code

Conclusions

The study of complex behaviours, such as facial expressions, would be limited without standardised systems that allow quantification of behaviour and cross-species comparisons. Facial Action Coding Systems (FACS) provide a means of addressing this problem, and by developing a FACS for the domestic horse (EquiFACS) we have produced a tool that allows the systematic recording of horse facial expressions in any context, with the potential to make direct comparisons across species. EquiFACS is an anatomically based, objective system that contains all the facial actions horses can possibly produce, making it suitable for many different research questions.

Horses have a rich facial repertoire, with 17 defined Action Units (AUs). Whilst this was less than the number of AUs humans display (27), it was slightly more than most other animals that FACS have been developed for (chimpanzees, 13, rhesus macaques, 13, orang-utans, 15, Hylobatids, 16, dogs, 16), with only cats displaying a larger facial repertoire (21, largely due to the extensive whisker and ear movements). The potential to make such cross-species comparisons can enhance our understanding of the meaning, function, and evolution of communicative behaviour [14, 44]. In fact, many of the horse

AUs were similar to the facial movements seen in other animals, including humans, chimpanzees, cats, and dogs. It was previously thought that humans possessed the most complex repertoire of facial expressions and that, in phylogenetic terms, the further away an animal was from humans, the more rudimentary their use of facial expressions would be. However, through the development of EquiFACS it is apparent that horses also have an extensive range of facial movements, sharing many Action Units with humans and other animals. This contributes to a growing body of evidence suggesting that the evolution of facial expressions was not driven entirely by phylogenetic pressures, but that other, socio-ecological factors had a significant influence [30, 35].

EquiFACS also provides those working in the horse community with a standardised language through which information can be shared, facilitating the investigation of questions relevant to horse management and welfare. It should be noted that whilst some of the video examples given with this manuscript contain recognisable horse behaviour (e.g. flehmen, Videos 25 and 30) when coding facial expressions, it is important not to try and immediately assess the overall behaviour, but instead to focus on describing the individual facial movements. One of the key actions seen in flehmen (AU122, the upper lip curl) has also been recorded in some pain expressions of the horse [45, 46], and yet is also seen in other, unrelated contexts (see Video 31). A potential application of EquiFACS would be to establish whether there are particular configurations of facial movements displayed alongside the upper lip curl (AU122), which help distinguish the different contexts.

While recent work has suggested that horses use apparently complex facial expressions [28, 47], and that certain facial movements are associated with pain in domestic horses [1, 2], until now the full capacity of horse facial expressions to convey a range of information has been largely overlooked. In particular, no studies have yet investigated whether there are facial expressions associated with positive experiences in horses – a critical yet poorly understood aspect of animal welfare [48]. EquiFACS can be applied to address this gap, with the potential to greatly facilitate future studies of horse welfare as well as extending our knowledge of equine communication and cognition.

Text S1. Detailed Dissection Protocol

Demographic information for the specimen was not available, however age was approximated from the teeth demonstrating that the specimen was an adult (circa 7 years). The tongue and larynx were removed, however the brain was still intact so the muscles of the cranial vault were undisturbed. The head was disarticulated from the neck at the atlanto-occipital joint. Where the head had been detached some of the cervical portion of the fascia was missing so the full extents of some of the ear muscles were unavailable. The head was obtained frozen and thawed in phosphate buffered saline solution prior to dissection.

The skin, superficial fasciae, and facial musculature were separated from the more deeply located muscles (the masseter and temporalis muscles) with a #21 scalpel blade and a variety of dissection tools. The right side of the face and superficial fascia were removed from the head first, beginning at the mandibular skin flap created when the head was removed from the body. The skin was removed from the inferior border of the mandible, and then a midline incision was made through the lower lip and the frontal and parietal regions. Finally the muscles of the external ear were released, and the external ear was removed with the skin. The levator labii superios proprius muscle was the only facial muscle to be left on the head. This procedure was then repeated for the left side of the face.

Care was taken to remove as much of the facial musculature as possible with the skin and superficial fasciae leaving behind only the bony attachments. This process created a "facial mask" for both the right and left side of the face that was separate from the skull and held all of the facial muscles except for the levator labii superious muscle, which was left behind with the skull. On the left side of the face the muscles of the lip (orbicularis oris, buccinator, and mentalis muscles) were also left on the skull, to give an alternative view of the relationships between the muscles and their relationship to the skull.

Once removed the face masks were examined before being fixed in 5% formalin solution, and initial impressions were recorded. Once fixed the right face mask was brought out and allowed to air dry for 30 minutes to allow the best differentiation

between the tissues, before the connective tissue was released from the musculature. This left the facial muscles discernable from the surrounding muscles and fasciae (see [1-4]).

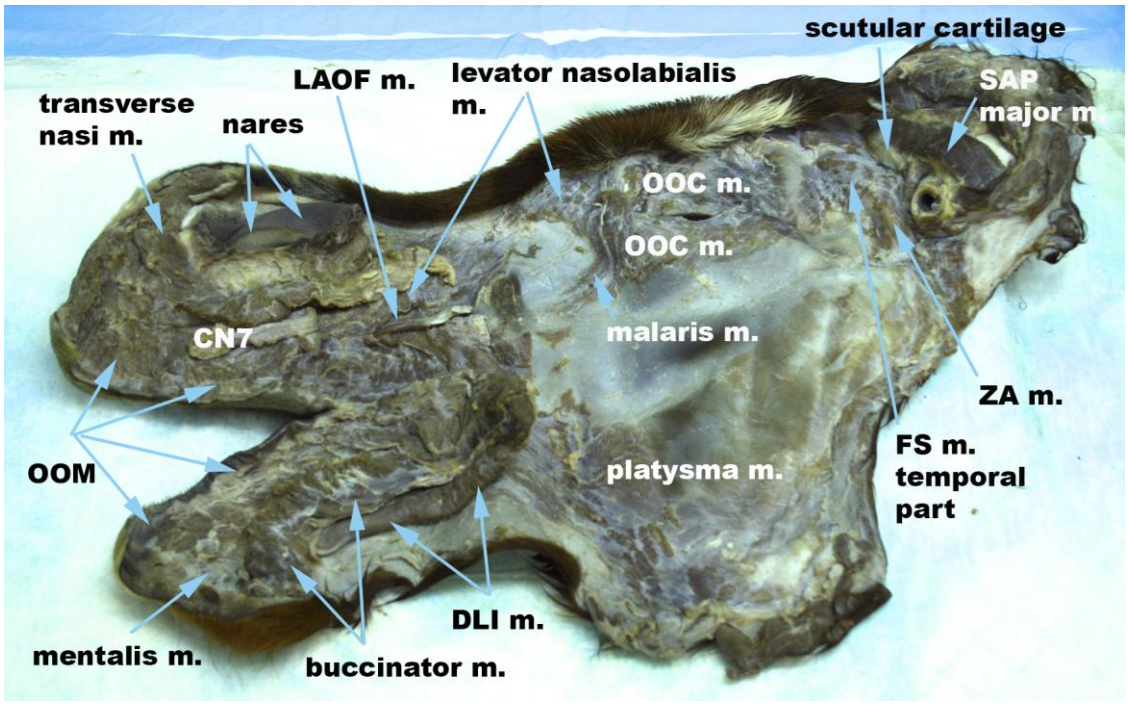


Figure 18. The facial mask from the right side of the face. The suffix ‘m.’ refers to muscle. OOM: orbicularis oris muscle. CN7: cranial nerve 7. LAOF m.: levator annuli oris fascialis muscle. OOC: orbicularis occuli muscle. SAP major m.: scutulo-auricularis profundus major muscle. ZAm.: zygomatico-auricularis muscle. FS m. temporal part: frontoscutularis muscle temporal part. DLI m.: depressor labii inferioris muscle.

Text S2. Detailed Descriptions of the Facial Muscles

See Figure 3 for an illustration of the facial muscles.

The eye region

Corrugator supercilii muscle

The corrugator supercilli muscle is a thin, small but distinct muscle immediately deep to the skin. It is attached to the root of the supraorbital process and to the skin of the upper eyelid, blending with the fibres of the orbicularis oculi muscle [1]. Although this muscle was reported in some texts (e.g. [1]) it was overlooked in others [2].

Levator anguli oculi medialis muscle

In our specimen this muscle presented as a robust band of longitudinal fibres within the orbicularis oculi muscle, rather than a distinct, separate muscle. The fibres attached to the base of the zygomatic process of the frontal bone and to the medial surface of the skin of the upper eyelid. This description was consistent with previous reports [2], however this muscle was omitted from other texts [1].

Orbicularis oculi muscle

This is a flat, elliptical sphincter muscle attached to the skin of the eyelids. This muscle is thin, but extensive, similar to the design in primates [3,4,5,6]. The portion of orbicularis oculi muscle surrounding the upper eyelid was substantially wider than the portion of the lower eyelid. The orbicularis oculi muscle is mostly attached to the skin of the eyelids, but some bundles are attached to the palpebral ligament at the medial canthus and to the lacrimal bone [1,2].

Levator palpebrae superioris muscle

This muscle technically belongs to the upper eyelid. We did not dissect the eye itself, and so could not locate this muscle; however, due to its presence in all investigated mammals, we expect that it would have been present [7].

Levator labii superioris alaeque nasi/levator nasolabialis muscle

This muscle is attached to the frontal and nasal bones. The deep section is attached to the upper lip and lateral wing of the nostril while the superficial section blends with the orbicularis oris muscle in the commissure of the lower lips [1,2] by common tendon with the levator labii superioris proprius muscle. It lies immediately deep to the skin [1], although splits and also has a deep section. In contrast to previous references, we found this muscle to be thick and robust [1].

The lower face

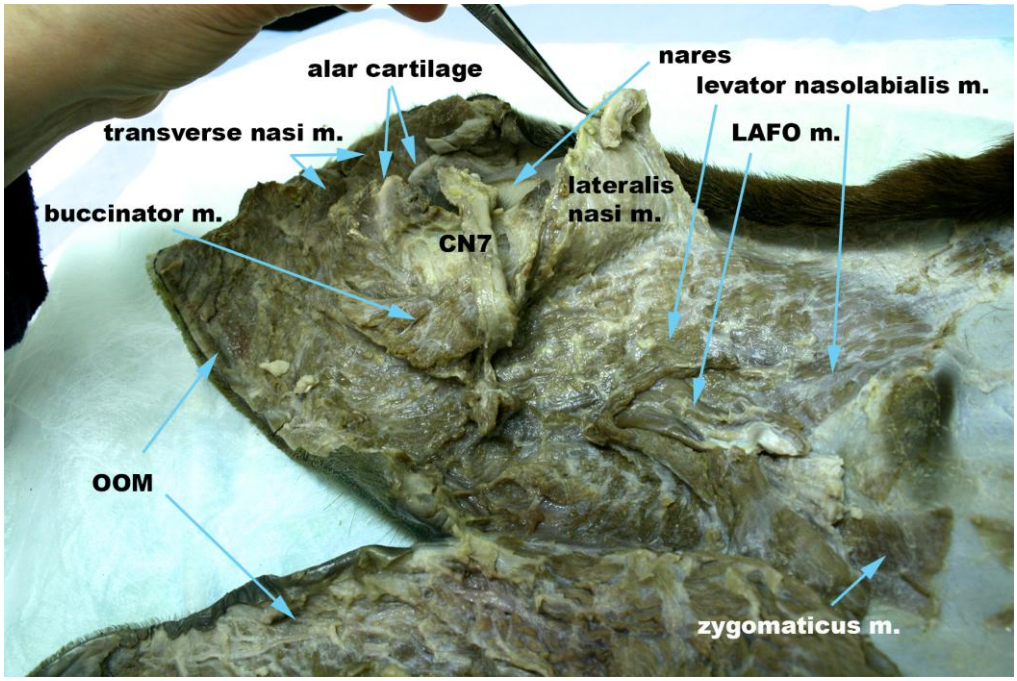


Figure 19. The muscles in the lips and midface of the horse. The suffix ‘m.’ refers to muscle. OOM: orbicularis oris muscle. CN7: cranial nerve 7. LAOF m.: levator annuli oris fascialis muscle.

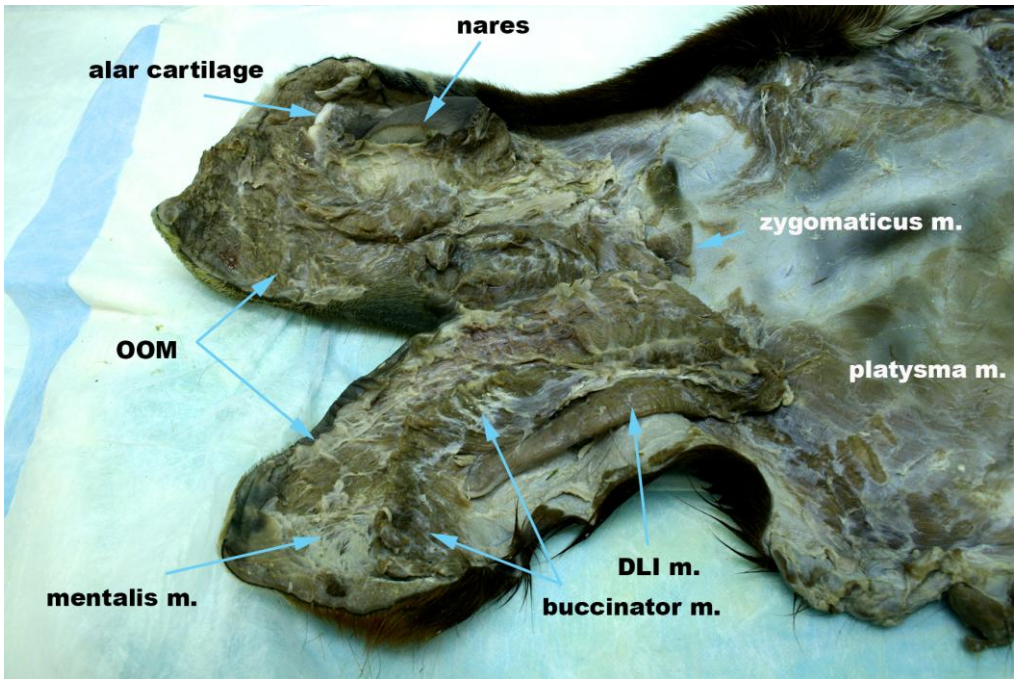


Figure 20. The muscles in the lower face of the horse. The suffix ‘m.’ refers to muscle. OOM: orbicularis oris muscle. DLI m.: depressor labii inferioris muscle.

Transverse nasi muscle

The transverse nasi muscle consists of transverse fibres and attaches to the alar cartilage. It was a very substantial muscle; a cross section taken was over 1cm thick. There were two sections, one that lay deep to the orbicularis oris and the lateralis nasi muscles, and one that lies superficial to the orbicularis oris muscle. This muscle was not reported in [2] and has no alternative name in the proposed standardised nomenclature, possibly due to its rarity among mammals [8].

Zygomaticus muscle

The zygomaticus muscle lies immediately deep to the skin of the cheek. It is a thin muscle, although clearly defined and thicker than the malaris or the platysma muscles in the horse. The zygomaticus muscle is attached to the fascia covering the masseter muscle below the facial crest, and to the corner of the lips, blending with the buccinator muscle [1,2].

It is generally reported that the horse only has one zygomaticus muscle. However, here we suggest that as in many primates the horse does have both a zygomatic major and a zygomatic minor muscle, and this previous misapprehension has been a result of inconsistent use of nomenclature. We propose that the zygomaticus muscle described here is equivalent to the zygomatic major muscle, and the malaris muscle described below is equivalent to the zygomatic minor muscle.

Levator annuli oris fascialis muscle (also called the caninus or dilator naris lateralis)

AMB reflected one head of the nasolabialis muscle so that we could see the full extent of the levator annuli oris fascialis muscle going to the base of the nostril. The levator annuli oris fascialis muscle was superficial and lay directly below the skin. It passed between the two branches of the levator nasolabials muscle [1,2], and lies superficial to the buccinator muscle. The levator annuli oris fascialis muscle originated in the rostral end of the facial crest and inserted into the lateral border of the nostril, with its lower fibres blending with the orbicularis oris muscle. The levator annuli oris fascialis muscle has previously been described as thin [1], however we found this muscle to be large, thick, and robust, particularly compared to previous primates dissected [3,4,5,6]. In line

with this we found that the levator annuli oris facialis muscle also went into a large and thick tendon.

Depressor labii inferioris muscle

This muscle lies on the lateral surface of the mandible along the ventral border of the buccinator muscle [1,2] and extends back along the mid-face to the masseter muscle. This was a large, smooth muscle with longitudinal fibres that attach to the lower lip with a tendon that spreads out, blending with the orbicularis oris and the depressor labii inferioris muscles of the opposite side [1,2].

Mentalis muscle

The mentalis muscle is small and located deep in the prominence of the mental region, ventral to the orbicularis oris muscle and rostral to the buccinator muscle. The fibres of the mentalis muscle arise from each side of the mandible and are inserted into the skin of the [1], running in an opposing direction to the fibres of the buccinator muscle. The buccinator and the mentalis muscles are separated by a fascial cleft. The mentalis muscle was omitted from one source that we referenced [2], and we found it to be more distinct than previously described [1].

Orbicularis oris muscle

This is a large, thick, and complex sphincter muscle that covers much of the lower face and extends well beyond the external corner of the lips, as far back as the rostral border of the masseter muscle. We also found a very large tendon in the orbicularis oris muscle that was not seen in the facial muscles of any of the other previous primates dissected [3,4,5,6], and have not found evidence of in other ruminants [1]. Tendons allow a greater specificity of movement and strengthen the muscle, in addition to being important in connecting muscle chains, so this tendon seems to support the complex muscle mass of the lower face in the horse.

The orbicularis oris muscle is much larger and thicker than seen in previous primate dissections including the chimpanzee, which was previously reported to have an unusually large orbicularis oris muscle. A cross section taken from the upper lip was approximately 3cm thick, the caudal section from near the masseter muscle was

approximately 2cm thick, and a cross section taken from the lower lip was approximately 1cm thick.

The orbicularis oris muscle contains the incisivus superior and inferior. These have previously been recorded as separate muscles [1] although here we consider them regions of specialization of the orbicularis oris, rather than specific muscles, as has been done previously in primates [7]. The orbicularis oris muscle is attached to numerous other facial muscles, including the buccinator, the dilator nares, and the transverse nasi muscles.

Malaris muscle

This muscle is very thin and lies directly below the skin, superficial to the zygomaticus muscle. Sisson [1] describes it as being variable across subjects (as it is in humans, chimpanzees, and rhesus macaques [5,6,9]), although we found it to be present and distinct in our specimen, and it has also been described in other sources [2]. The malaris muscle originates in the dorsal part of the facial crest blending with the skin posterior to the modiolus, and inserts into the lower eyelid [2].

The malaris muscle is a term commonly used to describe the zygomatic muscles of other species. The malaris muscle in the horse is seen to follow the same path as the zygomatic minor muscle in humans and other primates (stretching from the skin posterior to the corner of the mouth and blending into the orbicularis oculi muscle, as in humans)[5,6]. Therefore, we propose that the malaris muscle described here is equivalent to what is commonly termed the zygomatic minor muscle in primates.

The ear

The external ear comprises of the scutiform cartilage and the auricle (also called the pinna or conchal). The muscles of the ear were large and well defined. There were a number of clear, distinct muscles twirling around the ear, and forming windmill like shape around the scutiform cartilage. As the head was disarticulated from the neck of the atlanto-occipital joint the cervical portion of the skin was missing. This meant we were missing some muscles in the cervical region, and could not report the attachments of other muscles with confidence. Additionally, the instrinsic muscles (the anti tragicus

and the helicis) were not investigated as these are very small muscles that are confided to the auricle [1], and are not important in moving the external ear.

The muscles of the ear were arranged in a complex, multilayered fashion, with no evidence of the muscles acting like a sheet, as in many of the primates previously dissected, [3,4,5,6]. We also found a large fat pad at the base of the ear, and a large amount of adipose tissue surrounding the auricular muscles.

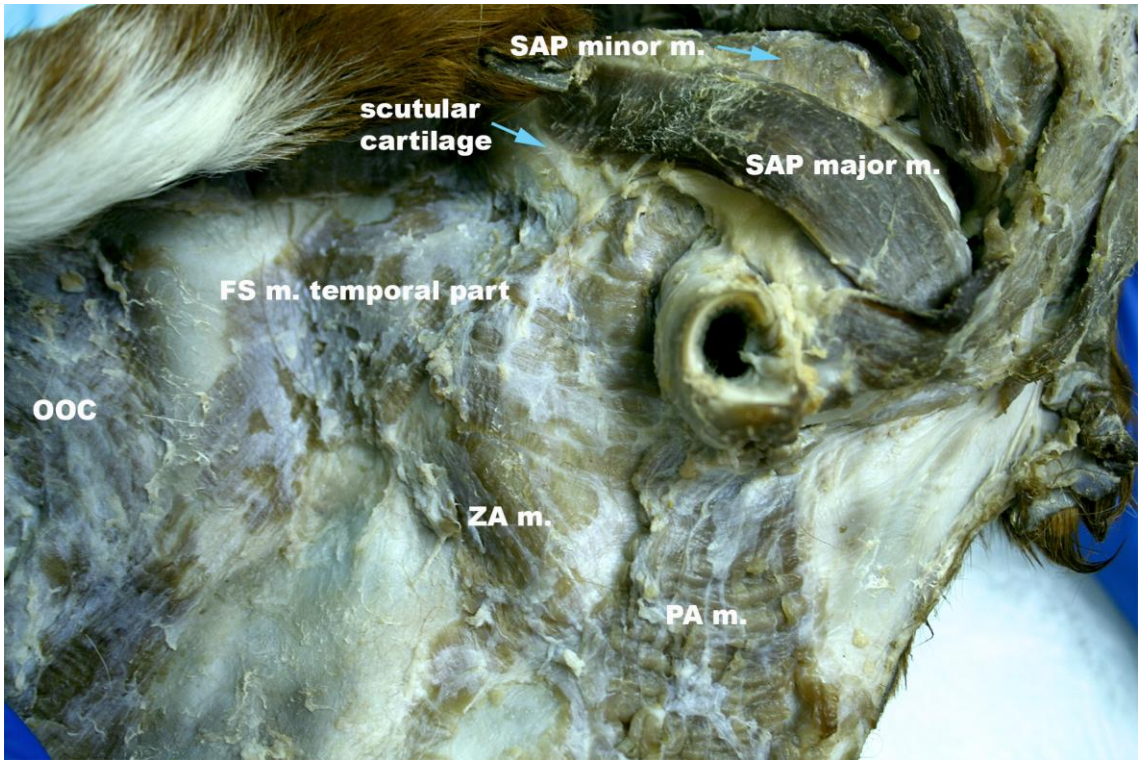


Figure 21. The muscles around the ear of the horse. The suffix ‘m.’ refers to muscle. OOC: orbicularis occuli muscle. FS m. temporal part: frontoscutularis muscle temporal part. SAP minor m.: scutulo-auricularis profundus minor muscle. SAP major m.: scutulo-auricularis profundus major muscle. PA m.: partoidoauricularis muscle. ZAm.: zygomatico-auricularis muscle.

Scutularis muscle

This is a thin muscular sheet situated subcutaneously over the temporalis muscle. Its fibres arise from the zygomatic arch and the frontal and parietal crests, and converge to the scutiform cartilage [1], which was a useful landmark for identification purposes. The scutularis muscle is divided into three: the interscutularis, the frontoscutularis, and the cervio-scutularis muscles.

Frontoscutularis muscle

This is further divided into two parts, the temporal and frontal parts. The temporal part arises from the zygomatic arch and the frontal part arises from the frontal crest. Both insert into the anterior border of the scutiform cartilage [1,2]. This was a thin band of muscle with fibres running longitudinally.

Interscutularis muscle (frontalis muscle)

The interscutularis muscle arises from the parietal crest, over which it is partially continuous with the muscle from the other side, and converges with the medial part of the scutiform cartilage [1,2]. This was a thin, flat sheet of muscle with transverse fibres. This muscle is thought to be termed the frontalis muscle in primates, and seems to be a relatively similar size to that seen in other primates [3,4,5,6].

Cervicoscutularis muscle

This was not well defined from the interscutularis muscle, and was also a thin flat sheet of muscle with transverse fibres. It arises from the nuchal crest and inserts into the medial border of the scutiform cartilage [1].

The anterior auricular muscles

Scutulo-auricularis superficialis muscle

This muscle arises on the scutiform cartilage and ends in the base of the conchal cartilage with the zygomatico-auricularis, the cervicoscutularis and the interscutularis muscles [1]. The scutulo-auricularis superficialis is listed as one muscle in some references, e.g. [2] but divided into three parts that are considered separately in others e.g. [1]. However, in order to keep the scutulo-auricularis profundus major and minor muscles intact we could not get a detailed view of the scutulo-auricularis superficialis muscles to examine this.

Zygomatico-auricularis muscle

This is a thin, broad sheet of muscle with lateral fibres that arise from the zygomatic arch and the parotid fascia, and insert ventro-medially on the auricle, partly above and partly below the insertion of the partido-auricularis muscle [1,2].

The dorso auricular muscles

Scutulo-auricularis superficialis accesorius muscle

The scutulo-auricularis superficialis accesorius muscle is a narrow band that arises from the posterior scutiform cartilage and the adjacent part of the superficial face of the scutiform cartilage, and is inserted into the surface of the conchal cartilage medial to the scutuloauricularis superior muscle with the two crossing each other at an acute angle [1]. This muscle was not reported in Budras et al. [2].

Scutulo-auricularis profundus major and minor muscles

These muscles are very deep, distinct, thick band that arise from the scutiform cartilage and insert into the auricle. The scutulo-auricularis profudus major muscle has been described as the strongest of the ear muscles [1], and we agree with this statement. It is flat and approximately 2.5cm wide.

Tragicus muscle

The tragicus muscle is a very small muscle that arises from the temporal bone just behind the acoustic process, and from the anular cartilage. It passes upwards to be inserted into the lower part of the anterior border of the conchar cartilage [1]. This muscle is not reported in Budras et al, [2], however we found it present as described in [1].

The posterior auricular muscles

The head used for this dissection was disarticulated from the neck of the atlantooccipital joint and we were missing the cervical region of the neck. Consequently, we could not confidently label the posterior auricular muscles. This included the cervico-auricularis superficialis, and the cervico auricularis profundus major and minor muscles [1,2].

Text S3. How to use the EquiFACS Manual

The EquiFACS manual lists the identified facial movements, accompanied by a description of the appearance changes and video illustrations. Where necessary, the proposed muscular basis of the actions is given and there are also sections on subtle differences between similar actions. Begin by reviewing these descriptions and video examples. Initially, it might seem impossible to distinguish the facial movements, but

just take time to review and reflect on the manual. Additionally, try and understand the muscular basis of the actions. When watching the video examples identify: the parts of the face that have moved and the direction of their movement; any wrinkles that have appeared or deepened; and the alterations in the shape and outline of the face.

Good knowledge of the neutral position of a horse's face is key. This is true for an individual horse and for horses in general. If you are unfamiliar with horses take some time to first observe a few horses in their relaxed state. See Figure 2 for some key facial landmarks of the horse Figure 3 for a guide to anatomical direction. Be aware that changes in camera position, lighting, and position of the horse's head can all influence the perception of the face. Be particularly cautious of coding in these situations, and only code an action where there is no doubt that it has occurred. This is especially applicable in non-standard situations, such as those where the horse is rubbing its face against something or chewing. These actions may cause the face to change shape, but not through any contraction of the facial muscles.

It is not necessary to be trained in any of the other FACS systems to be able to learn EquiFACS. However, as with all FACS systems EquiFACS requires certification to use. A test is available from www.equifacs.co.uk after obtaining a password from the EquiFACS team. If you have any questions about EquiFACS and how you might use it, or would like advice on coding or extra practice materials, please contact Jen Wathan (j.wathan@sussex.ac.uk).

Scoring techniques

When scoring a clip make an initial assessment of the action based on gut instinct and record this. Then go back and re-watch the clip, mark any queries you might have on the scoring sheet (e.g. AU 10 or 122?), and refer to the manual to help you resolve these. Review the clip and each part of the face (upper, ears, and lower) to see if you have omitted any possible actions, and check the miscellaneous codes and gross behaviours. Decide on a final score and list the AUs and ADs in numerical order.

Facial expressions are fluid and flexible behaviours. Your specific research question will determine exactly what to code. Generally expressions are coded as discrete

movements, and only the apex of an action is coded (the point of maximum expression). Actions are not counted as new events unless there is a complete return to neutral in between (e.g. in AU122, the upper lip curls and then the lips press together – a new action would be counted if the upper lip then curled again). When facial actions are slight it can be difficult to establish exactly which AU occurred. It can be that although the movement may by barely noticeable, there is still enough evidence for you to decide that a particular AU is present. However, if this is not the case then do not code any action.

With all animals it is likely that situations will occur where part of the face is obscured, and there are codes to denote these situations (see text S5). However, whereas with primates, dogs, and cats, the most useful information is captured from a frontal view, with horses the best position is an intermediate (3/4) view between frontal and profile. Consequently, it is likely that in most coding situations some information from one side of the face will be missing (the ears are an exception here, as both ears and their movements can usually be seen clearly regardless of the viewpoint). The visibility codes are not needed for these situations, but rather are used to reflect cases where the face is obscured in a way that influences coding, for example if the forelock is covering the horse's eyes.

We have not yet systematically investigated how symmetrical facial movements of horses are. However, from our observations it seems that a large number of facial movements are generally bilateral. For that reason, unless specifically interested in symmetry or laterality of facial expressions, we suggest coding movements as bilateral unless evidence of unilaterality can be clearly seen. Additionally, be cautious when coding any unilateral movements, as movements are only truly unilateral when there is absolutely no sign of the movement on the other side of the face. If there is even a trace of movement on the other side of the face, movements must be scored as bilateral. An 'L' or 'R', always coded from the perspective of the horse, can be used to prefix AUs that are genuinely unilateral.

If specifically interested in symmetry/laterality then it will be necessary to use two video cameras so that one camera can capture movements on each side of the face.

Visibility codes (text S5) can be used to denote which areas of the face are visible in each view, and which are not.

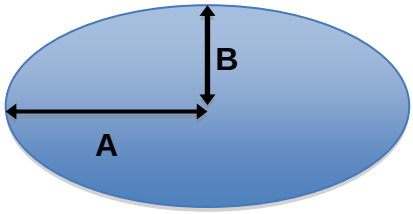
Text S4. Calculating the Percentage of Eye-white Visible (optional)

This method follows one used previously in cows [1-5]. The percentage of white of the total visible eye area can be calculated by measuring the area of the whole eye (T) and the area of the iris (including the pupil - I), then subtracting T from I, dividing this by T, and multiplying this value by 100 [(T-I/T) x100].

The most accurate way of doing this is to use computer software, such as SigmaScanPro, that calculates the area of a selected region. However, as this software is not available to all, a less accurate, but still effective, way is to treat the shape of the eye as an ellipse and calculate T and I by measuring the two orthogonal diameters of T and I, then plugging the values into the formula below. These measurements can be made by simply placing a ruler on the screen, or more accurately by drawing lines on the image in image manipulating software. They can be taken at time points to suit the specific research question and information required.

Formula for calculating the area of an ellipse:

Area = $\boldsymbol{\pi AB}$



The two areas can then be calculated by using the above formula (total eye: **T**; iris: **I**) and the percentage of white of the total visible eye (**PW**) can then be calculated as **PW** = 100 x ((**T**-**I**)/**T**).

Text S5. Miscellaneous Actions and Supplementary Codes

These describe additional facial actions that can influence the facial expression. For this reason, it is strongly recommended that the codes listed here are used. While AUs are identified in terms of their muscular basis, Action Descriptors (ADs) cover broader movements, or movements caused by vascular rather than muscular structures. For this reason, “Section A. Proposed muscular basis” has been omitted below.

Action Descriptor 19: tongue show

B. Appearance changes:

1. The tongue is shown and it reaches beyond the teeth.
2. The jaw must be lowered and the lips separated. Consequently, this AD is coded together with AU25+AU26 or AU25+AU27.
3. This often happens with a chewing action (AD81).

C. Minimum criteria to code AD19: the tongue is shown and it reaches beyond the teeth.

Action Descriptor 29: jaw thrust

B. Appearance changes:

1. The lower jaw is pushed forward (see Video 44).
2. The lower teeth extend in front of the upper teeth.

C. Minimum criteria to code AD29: the lower jaw moves forward.

Action Descriptor 30: jaw sideways

B. Appearance changes:

1. The lower jaw is moved sideways (see Video 44).
2. Chin and lower lip displaced from the midline to one side or the other.
3. If the mouth is open, the lower teeth appear to be off to one side.
4. If you wish to score the direction of jaw movement, prefix the code with an L (for left) or R (for right). Always code direction from the perspective of the horse.

C. Minimum criteria to code AD30: lateral displacement of the lower jaw so that it does not lie directly under the upper jaw.

Action Descriptor 133: blow

In humans AD33 describes air being blown out through the lips, expanding the cheeks. Horses do a similar action, but blow air out through the nostrils rather than the lips. To represent this difference, this action is labelled AD133 in horses.

B. Appearance changes:

1. Air is blown out sharply through the nostrils, often accompanied by an obvious noise (see Video 45).
2. The skin behind the nostril wing may stretch and expand.
3. This action will cause the nostrils to dilate briefly turning into a rounder shape. However, there is no need to code AD38, nostril dilator, unless the nostril dilation lasts longer than the blowing action, or if there is a specific interest in the dilation of the nostrils.

C. Minimum criteria to code AD133: air is blown sharply through the nostrils.

Action Descriptor 38: nostril dilator

B. Appearance changes:

1. The aperture of the nostril is increased (see Videos 46 and 47).
2. The nostril wings are flared and may bulge as the vascular network in the nare fills with blood.
3. The septum may be lifted and flared, and again may bulge (Video 47).
4. The shape of the nostril opening may change.
5. The nostril wings may be pushed forward.

C. Minimum criteria to code AD38: an increase in the aperture of the nostril.

Gross behaviour codes

Action Descriptor 50 – Vocalization

Action Descriptor 76 - Yawning

Action Descriptor 80 – Swallow

Action Descriptor 81 – Chewing

Action Descriptor 84 – Head shake side to side

Action Descriptor 85 – Head nod up and down

Action Descriptor 86 – Grooming

Action Descriptor 87 – Ear shake

Head movement codes

Changes in head position can influence the perception of the face, and may also contribute to the overall facial expression: for this reason head movement codes (ADs 51 – 58) are available to denote these changes. These codes should be applied to distinct shifts in orientation from a neutral position, where the horse has the head aligned with the body.

In previous FACS systems the ideal viewing perspective to code facial movements is from directly in front of the animal at face level, and so these codes were applicable from the coder's viewpoint. However, this is not always the ideal situation when coding the facial movements of horses. Often a perspective that is intermediate between frontal and profile views (3/4 view) is ideal, as this allows the coder to see the complex movements of the lip whilst also able to see movements of the nostrils and eyes. However, still try and use the codes to denote a change from the neutral position, even if not coding from that perspective.

N.B. Always code directions from the perspective of the subject being coded.

Action Descriptor 51: Head Turn Left

The head moves left along a vertical axis.

Action Descriptor 52: Head Turn Right

The head moves right along a vertical axis.

Action Descriptor 53: Head Up

The head moves upwards.

Action Descriptor 54: Head Down

The head moves downwards.

Action Descriptor 55: Head Tilt Left

The head is tilted to the left side (see Video 48).

Action Descriptor 56: Head Tilt Right

The head is tilted to the right side (see Video 48).

Action Descriptor 57: Nose Forward

The nose is pushed forward, and this is not due to a head toss (see Video 49).

Action Descriptor 58: Nose Back

The nose is brought in towards the chest. (see Video 50)

Eye movement codes

The extent to which horses can move their eyes to focus on stimuli is still unknown. For this reason, the eye movement codes 61-64 are not used in EquiFACS.

Visibility codes

These codes provide information about what areas of the face are not visible and able to be scored. In practical research situations, the face is often obscured, for example by the viewing angle, other animals, or the environment. These codes are a way to handle such situations. These codes can be used to distinguish times when no FACS codes are being scored because nothing is happening from no scores because the face or a part of the face cannot be seen.

Do not score an area of the face as "not visible" if it is possible to score any AU that affects the referenced area. Scores can be applied to only the left or the right side of the face if particularly interested in asymmetry by prefixing the code with L or R.

Visibility Code 70: Frontal Region Not Visible

The brow and frontal region (excluding the ears) cannot be seen.

Visibility Code 71: Eyes Not Visible

The eyes cannot be seen.

Visibility Code 72: Lower Face Not Visible

The lower face cannot be seen.

Visibility Code 73: Entire Face Not Visible

The entire face (including ears) is either out of view or cannot be clearly seen.

Visibility Code 74: Unscorable

This code is used when it appears there is something to code but for some reason (e.g. image out of focus) the movements cannot be clearly identified. This code is for the entire facial area and not for unilateral problems with visibility.

Visibility Code 75: Ears Not Visible

The ears cannot be seen. This can be suffixed by an L or an R to identify if only one ear is out of sight.

Supplemental Videos

Please contact j.wathan@sussex.ac.uk for the video files and their captions.

Acknowledgements

We would like to thank countless owners for letting us video their horses. Particular thanks must go to Sussex Horse Rescue Trust, Bowler's Riding School, and Little Park Farm. Katie Slocombe, Will Teasley and Becky Spooner helped with the collection of video data. We are grateful to Cátia Caeiro and Karen Schmidt for valuable advice, and also thanks to Cátia, Kate Grounds, Amy Smith, and Charlotte Lillis for help with the reliability coding. We must credit and thank Tim Smith for the anatomical drawing (Figure 3). Perry Habecker at the New Bolton Centre, University of Pennsylvania, sourced the specimen for the dissection. Emily Durham deserves a huge thank you for dealing with the specimen and thanks to Helen Spence for giving a second opinion on the age of the horse.

References

Main text

1. Gleerup KB, Forkman B, Lindegaard C, Andersen PH. An equine pain face. Vet Anaesth Analg. 2015;42(1):103-14. Epub 2014/08/02. doi: 10.1111/vaa.12212. PubMed PMID: 25082060.
2. Dalla Costa E, Minero M, Lebelt D, Stucke D, Canali E, Leach MC. Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration. PLoS one. 2014;9(3):e92281. doi: 10.1371/journal.pone.0092281.
3. Ekman P, Friesen WV, Hager JC. Facial Action Coding System. Salt Lake City: Research Nexus; 2002.
4. Diogo R, Wood BA, Aziz MA, Burrows A. On the origin, homologies and evolution of primate facial muscles, with a particular focus on hominoids and a suggested unifying nomenclature for the facial muscles of the Mammalia. Journal of Anatomy. 2009;215(3):300-19. Epub 2009/06/18. doi: 10.1111/j.1469-7580.2009.01111.x. PubMed PMID: 19531159; PubMed Central PMCID: PMC2750763.
5. Bruce V, Young A. Face Perception: Taylor & Francis; 2013.
6. Hole G, Bourne V. Face Processing: Psychological, Neuropsychological, and Applied Perspectives: OUP Oxford; 2010.
7. Waller BM, Bard KA, Vick SJ, Smith Pasqualini MC. Perceived differences between chimpanzee (Pan troglodytes) and human (Homo sapiens) facial expressions are related to emotional interpretation. Journal of Comparative Psychology. 2007;121(4):398-404. Epub 2007/12/19. doi: 10.1037/0735-7036.121.4.398. PubMed PMID: 18085923.
8. Vick SJ, Waller BM, Parr LA, Smith Pasqualini MC, Bard KA. A Cross-species Comparison of Facial Morphology and Movement in Humans and Chimpanzees Using the Facial Action Coding System (FACS). J Nonverbal Behav. 2007;31(1):1-20. Epub 2007/03/01. doi: 10.1007/s10919-006-0017-z. PubMed PMID: 21188285; PubMed Central PMCID: PMC3008553.
9. Caeiro CC, Waller BM, Zimmermann E, Burrows AM, Davila-Ross M. OrangFACS: A Muscle-Based Facial Movement Coding System for Orangutans (Pongo spp.). International Journal of Primatology. 2012;34(1):115-29. doi: 10.1007/s10764-012-9652-x.
10. Parr LA, Waller BM, Burrows AM, Gothard KM, Vick SJ. MaqFACS: A muscle-based facial movement coding system for the rhesus macaque. Am J Phys Anthropol. 2010;143(4):625-30. Epub 2010/09/28. doi: 10.1002/ajpa.21401. PubMed PMID: 20872742; PubMed Central PMCID: PMC2988871.
11. Waller BM, Lembeck M, Kuchenbuch P, Burrows AM, Liebal K. GibbonFACS: A Muscle-Based Facial Movement Coding System for Hylobatids. International Journal of Primatology. 2012;33(4):809-21. doi: 10.1007/s10764-012-9611-6.
12. Waller BM, Peirce K, Caeiro CtC, Scheider L, Burrows AM, McCune S, et al. Paedomorphic Facial Expressions Give Dogs a Selective Advantage. PLoS one. 2013;8(12):e82686. doi: 10.1371/journal.pone.0082686.
13. Caeiro CC, Burrows, A.M., Waller, B.M. CatFACS. Univeristy of Portsmouth; 2013.
14. Waller BM, Misch A, Whitehouse J, Herrmann E. Children, but not chimpanzees, have facial correlates of determination. Biol Lett. 2014;10(3):20130974.

Epub 2014/03/07. doi: 10.1098/rsbl.2013.0974. PubMed PMID: 24598107; PubMed Central PMCID: PMC3982434.
15. Outram AK, Stear NA, Bendrey R, Olsen S, Kasparov A, Zaibert V, et al. The Earliest Horse Harnessing and Milking. Science. 2009;323(5919):1332-5. doi: 10.1126/science.1168594.
16. Clutton-Brock J. A Natural History of Domesticated Mammals: Cambridge University Press; 1999.
17. Hare B, Brown M, Williamson C, Tomasello M. The domestication of social cognition in dogs. Science. 2002;298(5598):1634-6. Epub 2002/11/26. doi: 10.1126/science.1072702. PubMed PMID: 12446914.
18. Trut L. Early canid domestication: The farm-fox experiment. American Scientist. 1999;87(2):160-9.
19. Hare B, Plyusnina I, Ignacio N, Schepina O, Stepika A, Wrangham R, et al. Social cognitive evolution in captive foxes is a correlated by-product of experimental domestication. Curr Biol. 2005;15(3):226-30. Epub 2005/02/08. doi: 10.1016/j.cub.2005.01.040. PubMed PMID: 15694305.
20. Waring GH. Horse Behavior: Noyes Publications/William Andrew Pub.; 2003.
21. Aureli F, Schaffner Colleen M, Boesch C, Bearder Simon K, Call J, Chapman Colin A, et al. Fission-Fusion Dynamics: New Research Frameworks. Current Anthropology. 2008;49(4):627-54. doi: 10.1086/586708.
22. Hinde RA. A conceptual framework. In: Hinde RA, editor. Primate social relationships;an integrated approach. Oxford, UK: Blackwells; 1983. p. 1-7.
23. Harman AM, Moore S, Hoskins R, Keller P. Horse vision and an explanation for the visual behaviour originally explained by the 'ramp retina'. Equine Veterinary Journal. 1999;31(5):384-90. doi: 10.1111/j.2042-3306.1999.tb03837.x.
24. Timney B, Keil K. Visual acuity in the horse. Vision Res. 1992;32(12):2289-93. Epub 1992/12/01. PubMed PMID: 1288005.
25. Miller PE, Murphy CJ. Vision in dogs. J Am Vet Med Assoc. 1995;207(12):1623-34. Epub 1995/12/15. PubMed PMID: 7493905.
26. McDonnell S. The Equid Ethogram: A Practical Field Guide to Horse Behavior: ECLIPSE Press; 2003.
27. Schilder MBH, van Hooff JARAM, van Geer-Plesman CJ, Wensing JB. A Quantitative Analysis of Facial Expression in the Plains Zebra. Zeitschrift für Tierpsychologie. 1984;66(1):11-32. doi: 10.1111/j.1439-0310.1984.tb01352.x.
28. Klingel H. Communication in Perissodactyla. In: Sebeok TA, editor. How Animals Communicate. Bloomington & London: Indiana University Press; 1977.
29. Parr LA, Waller BM, Vick SJ, Bard KA. Classifying chimpanzee facial expressions using muscle action. Emotion. 2007;7(1):172-81. Epub 2007/03/14. doi: 10.1037/1528-3542.7.1.172. PubMed PMID: 17352572; PubMed Central PMCID: PMC2826116.
30. Waller BM, Micheletta J. Facial Expression in Nonhuman Animals. Emotion Review. 2013;5(1):54-9. doi: 10.1177/1754073912451503.
31. Ashdown RR, Done SH. Color Atlas of Veterinary Anatomy, Volume 2, The Horse: Elsevier Health Sciences UK; 2011.
32. Burrows AM, Smith TD. Muscles of facial expression in Otolemur, with a comparison to lemuroidea. Anat Rec A Discov Mol Cell Evol Biol. 2003;274(1):827-36. Epub 2003/08/19. doi: 10.1002/ar.a.10093. PubMed PMID: 12923893.
33. Burrows AM, Waller BM, Parr LA, Bonar CJ. Muscles of facial expression in the chimpanzee (Pan troglodytes): descriptive, comparative and phylogenetic contexts. Journal of Anatomy. 2006;208(2):153-67. doi: 10.1111/j.1469-7580.2006.00523.x.

34. Burrows AM, Waller BM, Parr LA. Facial musculature in the rhesus macaque (Macaca mulatta): evolutionary and functional contexts with comparisons to chimpanzees and humans. Journal of Anatomy. 2009;215(3):320-34. doi: 10.1111/j.1469-7580.2009.01113.x.
35. Burrows AM, Diogo R, Waller BM, Bonar CJ, Liebal K. Evolution of the Muscles of Facial Expression in a Monogamous Ape: Evaluating the Relative Influences of Ecological and Phylogenetic Factors in Hylobatids. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology. 2011;294(4):645-63. doi: 10.1002/ar.21355.
36. Budras KD, Sack WO, Rock S, Horowitz A, Berg R. Anatomy of the Horse: with Aaron Horowitz and Rolf Berg. 6th ed. Hanover, Germany: Schlutersche Verlagsgesellschaft mbH & Co. KG; 2012.
37. Sisson S, Grossman JD. The Anatomy of the Domestic Animals. 4th ed. Philadelphia and London: W. B. Saunders company; 1962.
38. Ekman P, Friesen WV, Hager JC. Facial Action Coding System Investigator's Guide. Salt Lake City, USA: Research Nexus; 2002.
39. Gregory WK. Our Face from Fish to Man: A Portrait Gallery of Our Ancient Ancestors and Kinsfolk Together with a Concise History of Our Best Features: G.P. Putnam's Sons; 1929.
40. Huber E. Evolution of facial musculature and expression. Baltimore: John Hopkins Press; 1931.
41. Land MF, Nilsson DE. Animal Eyes: Oxford University Press; 2002.
42. Sandem A-I, Braastad BO. Effects of cow–calf separation on visible eye white and behaviour in dairy cows—A brief report. Applied Animal Behaviour Science. 2005;95(3-4):233-9. doi: 10.1016/j.applanim.2005.04.011.
43. Whalen PJ, Kagan J, Cook RG, Davis FC, Kim H, Polis S, et al. Human Amygdala Responsivity to Masked Fearful Eye Whites. Science. 2004;306(5704):2061. doi: 10.1126/science.1103617.
44. Tinbergen N. On aims and methods of ethology. Zeitschrift für Tierpsychologie. 1963;20(4):410-33.
45. Bussieres G, Jacques C, Lainay O, Beauchamp G, Leblond A, Cadore JL, et al. Development of a composite orthopaedic pain scale in horses. Research in Veterinary Science. 2008;85(2):294-306. Epub 2007/12/07. doi: 10.1016/j.rvsc.2007.10.011. PubMed PMID: 18061637.
46. Jochle W, Moore JN, Brown J, Baker GJ, Lowe JE, Fubini S, et al. Comparison of detomidine, butorphanol, flunixin meglumine and xylazine in clinical cases of equine colic. Equine Veterinary Journal. 1989;21(S7):111-6. doi: 10.1111/j.2042-3306.1989.tb05668.x.
47. Wathan J, McComb K. The eyes and ears are visual indicators of attention in domestic horses. Curr Biol. 2014;24(15):R677-9. Epub 2014/08/06. doi: 10.1016/j.cub.2014.06.023. PubMed PMID: 25093554; PubMed Central PMCID: PMC4123162.
48. Boissy A, Manteuffel G, Jensen MB, Moe RO, Spruijt B, Keeling LJ, et al. Assessment of positive emotions in animals to improve their welfare. Physiology & Behavior. 2007;92(3):375-97. Epub 2007/04/13. doi: 10.1016/j.physbeh.2007.02.003. PubMed PMID: 17428510.

Text S1 References

1. Burrows AM, Diogo R, Waller BM, Bonar CJ, Liebal K. Evolution of the Muscles of Facial Expression in a Monogamous Ape: Evaluating the Relative Influences of Ecological and Phylogenetic Factors in Hylobatids. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology. 2011;294(4):645-63. doi: 10.1002/ar.21355.
2. Burrows AM, Smith TD. Muscles of facial expression in Otolemur, with a comparison to lemuroidea. Anat Rec A Discov Mol Cell Evol Biol. 2003;274(1):827-36. Epub 2003/08/19. doi: 10.1002/ar.a.10093. PubMed PMID: 12923893.
3. Burrows AM, Waller BM, Parr LA. Facial musculature in the rhesus macaque (Macaca mulatta): evolutionary and functional contexts with comparisons to chimpanzees and humans. Journal of Anatomy. 2009;215(3):320-34. doi: 10.1111/j.1469-7580.2009.01113.x.
4. Burrows AM, Waller BM, Parr LA, Bonar CJ. Muscles of facial expression in the chimpanzee (Pan troglodytes): descriptive, comparative and phylogenetic contexts. Journal of Anatomy. 2006;208(2):153-67. doi: 10.1111/j.1469-7580.2006.00523.x.

Text S2 References

1. Sisson S, Grossman JD. The Anatomy of the Domestic Animals. 4th ed. Philadelphia and London: W. B. Saunders company; 1962.
2. Budras KD, Sack WO, Rock S, Horowitz A, Berg R. Anatomy of the Horse: with Aaron Horowitz and Rolf Berg. 6th ed. Hanover, Germany: Schlutersche Verlagsgesellschaft mbH & Co. KG; 2012.
3. Burrows AM, Diogo R, Waller BM, Bonar CJ, Liebal K. Evolution of the Muscles of Facial Expression in a Monogamous Ape: Evaluating the Relative Influences of Ecological and Phylogenetic Factors in Hylobatids. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology. 2011;294(4):645-63. doi: 10.1002/ar.21355.
4. Burrows AM, Smith TD. Muscles of facial expression in Otolemur, with a comparison to lemuroidea. Anat Rec A Discov Mol Cell Evol Biol. 2003;274(1):827-36. Epub 2003/08/19. doi: 10.1002/ar.a.10093. PubMed PMID: 12923893.
5. Burrows AM, Waller BM, Parr LA. Facial musculature in the rhesus macaque (Macaca mulatta): evolutionary and functional contexts with comparisons to chimpanzees and humans. Journal of Anatomy. 2009;215(3):320-34. doi: 10.1111/j.1469-7580.2009.01113.x.
6. Burrows AM, Waller BM, Parr LA, Bonar CJ. Muscles of facial expression in the chimpanzee (Pan troglodytes): descriptive, comparative and phylogenetic contexts. Journal of Anatomy. 2006;208(2):153-67. doi: 10.1111/j.1469-7580.2006.00523.x.
7. Diogo R, Wood BA. Comparative Anatomy and Phylogeny of Primate Muscles and Human Evolution: Science Publishers; 2012.
8. Diogo R, Wood BA, Aziz MA, Burrows A. On the origin, homologies and evolution of primate facial muscles, with a particular focus on hominoids and a suggested unifying nomenclature for the facial muscles of the Mammalia. Journal of Anatomy. 2009;215(3):300-19. Epub 2009/06/18. doi: 10.1111/j.1469-7580.2009.01111.x. PubMed PMID: 19531159; PubMed Central PMCID: PMC2750763.
9. Waller BM, Cray JJ, Burrows AM. Selection for universal facial emotion. Emotion. 2008;8(3):435-9. Epub 2008/06/11. doi: 10.1037/1528-3542.8.3.435. PubMed PMID: 18540761.

Text S4 References

1. Sandem AI, Janczak AM, Braastad BO. A short note on effects of exposure to a novel stimulus (umbrella) on behaviour and percentage of eye-white in cows. Applied Animal Behaviour Science. 2004;89(3-4):309-14. doi: 10.1016/j.applanim.2004.06.011.
2. Sandem AI, Braastad BO, Bøe KE. Eye white may indicate emotional state on a frustration-contentedness axis in dairy cows. Applied Animal Behaviour Science. 2002;79(1):1-10. doi: http://dx.doi.org/10.1016/S0168-1591(02)00029-1.
3. Sandem AI, Janczak AM, Salte R, Braastad BO. The use of diazepam as a pharmacological validation of eye white as an indicator of emotional state in dairy cows. Applied Animal Behaviour Science. 2006;96(3-4):177-83. doi: 10.1016/j.applanim.2005.06.008.
4. Sandem AI, Braastad BO. Effects of cow–calf separation on visible eye white and behaviour in dairy cows—A brief report. Applied Animal Behaviour Science. 2005;95(3-4):233-9. doi: 10.1016/j.applanim.2005.04.011.
5. Core S, Widowski T, Mason G, Miller S. Eye white percentage as a predictor of temperament in beef cattle. Journal of Animal Science. 2009;87(6):2168-74. Epub 2009/02/14. doi: 10.2527/jas.2008-1554. PubMed PMID: 19213718.

Article II

Auditory-Motor Representation for the Transfer of Emotional Information in the Horse

Wathan, J. & McComb, K. *In the style of Biology Letters.*

Abstract

In humans, hearing affective non-verbal vocalisations activates sensorimotor regions that are also activated during the production of emotional facial expressions. This audio-motor mirror system plays a key role in auditory and visual emotion recognition, a process that is clearly fundamental for modulating interpersonal reactivity. Previously, this multi-modal mirroring of emotional information had not been seen in any non-human species. Here we use a novel experimental paradigm to show that emotional conspecific vocalisations elicit the production of corresponding facial expressions in domestic horses (*Equus caballus*) in the absence of any visual input. These findings demonstrate the first evidence of audio-motor representation for the transfer of emotional information in a non-human animal. This system offers a physiological mechanism for gaining and representing social information, and the presence of such a mechanism in the horse suggests that it is either an ancestrally conserved system or that it has evolved convergently in social species.

Introduction

Perception-action representations are considered critical mechanisms underpinning the understanding of others' actions (1-3). In humans and macaques, neurons have been identified that fire selectively both when performing an action and upon seeing an action performed by another (mirror neurons - 1, 3, 4). Auditory-motor mirror neurons have also been identified in macaques and swamp sparrows, which respond selectively to the auditory perception of an action and the manual performance of the same action (1, 2, 4). In visual observation a correspondence could be established between low-level visual inputs and motor representations in the premotor cortex, however, auditory signals provide much less information with which to establish a correspondence with a motor representation of an action (5). Therefore it has been suggested that auditory-motor representations indicate multi-modal integration of a more complex level than visual-motor mapping.

In humans, perceiving another's emotional expressions, whether visual (facial expressions) or auditory (non-verbal vocalisations), activates sensorimotor regions that are involved in the production of emotional facial actions (6-9). Disruption of these sensorimotor areas interferes with the ability to recognise emotional expressions across modalities, suggesting that such multi-modal perception-action representations are critical for emotion discrimination (10-12). Similarly, the perception of an emotional expression can also cause automatic mimicry of facial and body movements (potentially a by-product of these representations), and inhibiting this mimicry interferes with the ability to process emotional information (13, 14). While multi-modal representations of this sort could offer a general mechanism for emotion recognition and the transfer of emotional information in other species too, whether they exist in non-human animals is still not known.

We used a systematic and anatomically based method of coding facial expressions (the Equine Facial Action Coding System - EquiFACS) to document the facial expressions horses produced when giving two affective vocalisations: the squeal (a vocalisation given in negative contexts) and the nicker (a vocalisation given in positive contexts). We then used playback experiments to present these calls to horses, and recorded their facial and behavioural responses.

Methods

Study animals

Thirty-two horses were recruited from a riding school in East Sussex, UK. Subjects included 10 mares and 22 gelded males, ages ranged from 5 to 24 years (M = 16.66 *SD* = 4.47), and all horses were naïve to the experimental set up.

Call acquisition

Eight good quality recordings, 4 nickers and 4 squeals (all from different call providers), were selected from a library of previously recorded vocalisations to make the final stimuli set. The call providers of the squeals were all adult males (aged 7 and 13 years, two ages unknown); one adult male plus three adult females (aged 5, 6, 11, and approx. 30 years) provided the nickers. These were normalised to 95% peak intensity. Recordings had been made opportunistically using a Sennheisser directional microphone with a windshield attached (models MKH816, MK416, ME66) linked to a digital audio recorder (Tascam HD-P2/MKH416 Marantz PMD660) or Uher tape recorder. Calls were recorded in mono at distances between 1 and 30m, with a sampling frequency of 48kHz and a sampling width of 24bit. Calls recorded onto cassette tape were digitised using a Marantz PMD660 connected to a MacBook pro via an iMic adaptor.

Playback procedure

Experiments were carried out in a fenced outdoor area in August/September 2014 (Figure 22). Horses were given two trials separated by at least seven days (*M*=11.50, *SD*=4.19, max=19). Each playback consisted of one vocalisation from one horse, which was played twice separated by a 10s interval, and followed by 2 minutes of silence. The speaker was placed 7m outside the fenced area, so that the fence prevented horses entering the immediate vicinity of the speaker, and disguised in thick natural vegetation. A wooden pole (4m long) was placed on the ground to act as a positioning marker, 15m away from the speaker.

Horses were held by a handler on a loose lead rope during trials. The handler stood on the left side of the horse, as is traditional in Britain, but stood facing towards the

horse's tail, so they could not provide any facial feedback. The handler wore small earpiece headphones and listened to white noise from a hand held MP3 player for the duration of the trial, to mask the sound of the playback. The handler was therefore blind to the stimuli presentation and could not incidentally cue the horse or create a 'Clever Hans Effect.'

Calls were played from a Mipro MA707 powered speaker connected to a MacBook using an IMic adaptor. Nickers were played at 90db peak intensity at 1m from source and the squeals at 100db.The order in which the vocalisations were played were counterbalanced across trials. Trials were recorded by two video cameras partially hidden behind a barrier 10m from the starting position of the horses. One video camera (Panasonic HC-V720) recorded a close up of the facial behaviour and the other (Panasonic HC-X920) recorded a full body view.

Figure 22. Example of the experimental set up. The speaker is hidden in the natural vegetation surrounding the testing area. In order to eliminate any possibility of a 'Clever Hans Effect' the handler is facing the rear of the horse and wearing headphones so she is blind to the stimulus that was presented.

Production of facial expressions during calls

In order to investigate the normal facial expressions associated with the production of squeals and whinnies we obtained six video clips of squeals and six video clips of nickers that were recorded ad-hoc. Within these clips there were often multiple vocalisations so a third party chose the specific calls for analysis, based on clarity of the facial area during the vocalisation. The final stimuli set were coded using the Equine Facial Action Coding System (EquiFACS) by JW.

Behavioural analysis and measurement of facial expressions

Videos were analysed for behavioural responses using Sportscode Gamebreaker Plus (www.sportstech.com). All videos were analysed blind to the vocalisation that was played in the trial (a third party identified the start and end times of each trial and then the videos could be coded with the sound track off). Detailed behavioural responses (including facial responses) were coded from the onset of the first playback to 10s after the end of the second playback. Prolonged looking was analysed for the duration of the whole trial. See Table 3 for definitions of the behaviours used for coding. Facial expressions were coded using the EquiFACS by two qualified coders (JW and KG). Facial expressions were not coded during vocalisations or chewing.

Ten videos (16%) were double coded and reliability was assessed using Spearman's rho correlation for continuous variables and Crohnbach's alpha for categorical variables, FACS reliability was assessed using Wexler's ratio (15). This revealed good to excellent levels of agreement for both the EquiFACS coding (ear movements = 0.78; eye movements = 0.82; lower face movements = 0.75) and the behavioural coding (looking time/direction = 0.86, $p<0.0001$; frozen time = 0.95, $p<0.0001$; approach time = 1, $p<0.0001$; avoid time = 1, $p<0.0001$; ear position = 0.92, $p<0.0001$; prolonged looking = 0.9; direction of first gaze bias = 1).

Statistical analysis

All continuous variables were analysed using Wilcoxon Signed-Rank test. Whether horses displayed prolonged looking was analysed using McNemar's test. Direction of first head turn was analysed using two-tailed binomial probabilities. Horses were excluded from the analysis of a facial action if the variable could not be coded for

more than 50% of the trial length (e.g. if area of the face was out of sight). A further horse was excluded from analysis of all eye movements because he was subsequently found to have an eye infection during the testing. No horses were excluded from the behavioural analysis.

Table 3. Definitions of behaviours used for behavioural video coding.

Behaviour		Definition
Looking at speaker		Time spent with head orientated directly towards the location of the speaker
Gaze bias left/right		Oriented to the speaker (judged by at least one ear still oriented towards the speaker location) but with the head turned to one side. Gaze bias left refers to a preference for viewing with the left eye (i.e. head turn right) and gaze bias right a preference for the right eye (head turn left).
Avoidance behaviour		Horse moves away from the speaker with visible alarm, e.g. nostril flaring, wide eyes, tense muscles.
Approach behaviour		Horse reaches their nose or moves their body forward towards the speaker.
Prolonged looking		Horse is continues to be attentive and looks at the speaker for 30s consecutively.
Frozen		Horse is looking at the speaker but completely still.
Both ears forward		The openings of both ears are facing forward of the midline.
Both ears back		The openings of both ears are facing posteriorly of the midline.
Asymmetrical ears	Left ear forward/right ear back	The opening of the left ear is facing forward of the midline while the right ear opening is directed posteriorly.
	Right ear forward/left ear back	The opening of the right ear is facing forward of the midline while the left ear opening is directed posteriorly.

Results

Facial Actions produced when vocalising

Full FACS coding of the vocalisations is seen in Table 4. Key facial actions appeared in at least 50% of clips coded. For the squeal these were: EAD103, ears flattener, EAD104, ears rotator, AU18, lip pucker, AU17, chin raiser, and AHU13, nostril lift, AU101, eyebrow raiser, AU5, lid raiser, AD1, increased eye white, and AU12, lip stretcher. Key facial actions produced when nickering were: AU101, eyebrow raiser, AU47, half blink, AU18, lip pucker, and EAD104, ears rotator. Although AU18, lip pucker, was present in the production of both vocalisations, it was presented quite differently: when producing squeals it was characterised by strong actions of AU18 that were typically held on the face, whereas in nickers it was characterised by milder, short repetitive actions.

Facial actions produced when hearing vocalisations

When horses heard a squeal, they were significantly more likely to produce the facial actions that are displayed when producing a squeal. Specifically, this included AUH13, the nostril lift, AU18, the lip pucker, AU5, the lid raiser. As AU18 was displayed in the production of both nickers and squeals yet was held on the face for longer in the production of squeals, we also analysed the time that horses displayed AU18 for in our playbacks. This showed that horses held AU18 on their face for longer in response to hearing the squeal than the nicker. There were more horses that produced higher rates of AU47, the half blink, to the nicker than the squeal, however this difference was not significantly different (Table 5).

Behavioural responses

Horses were generally interested in the playbacks, and spent most of the trial looking in the direction of the speaker; although there was no difference in the time that they spent looking at the speaker in response to the nicker (*Mdn* = 18.17s) and the squeal (*Mdn* = 18.22s), Z=0.43, p=0.67. However, upon hearing the squeal horses were significantly more likely to show a left gaze bias (*Mdn* = 0.75s) than a right gaze bias (*Mdn* =0s), Z=2.84, p=0.005. Similarly, of the 22 horses who demonstrated a head turn after

Table 4. Facial actions produced when vocalising, as measured with the Equine Facial Action Coding System (EquiFACS) see (16) for full descriptions and examples of the codes. Key facial actions (appeared in >50% of clips) are highlighted in bold. In some clips visibility too poor to code certain actions – these are listed where applicable.

Call produced	**Facial actions produced**	**Facial actions not scored**
Squeals	**AD1** + **AU5** + **AUH13** + **AU17** + **AU18** + AU145 + **EAD103** + **EAD104**	
	AU12 + **AUH13** + **AU17** + **AU18** + EAD101+ **EAD103** + **EAD104**	AU101, AU5, AD1
	AU47 + **AUH13** + **EAD103** + **EAD104**	AU101, AU5, AD1, AU17
	AU101 + **AD1** + **AU12** + **AUH13** + **AU17** + **AU18** + **EAD103** + **EAD104**	
	AU101 + **AD1** + **AU5** + **AU12** + **AUH13** + AU16 + **AU17** + **AU18** + **EAD103** + **EAD104**	
	AU101 + **AD1** + **AU5** + **AUH13** + **AU17** + **AU18** + **EAD103** + **EAD104**	AUH13, AU12
Nickers	**AU101** + **AU47** + AU16 + **AU18** + EAD101 + EAD102 (R) + **EAD104 (L)**	
	AD1 + **AU47** + **AU101** + **AU18** + **EAD104**	
	AU101 + AU47 + AU18	
	AU101 + AU47 + AU18 + EAD104	
	AU101 + **AU47** +**AU18**	
	AU101 + AD1 + AU5 + **AU47** + **AU18**	

Table 5. Facial actions produced in response to hearing a vocalisation. Significant differences between the rate of actions produced when hearing the squeal and the nicker are highlighted in bold and denoted with an asterisk; s>n reports the number of cases where the action was produced more in response to the squeal than the nicker, s<n a lower rate in response to the squeal than the nicker, and s=n denotes equal response rate to the squeal and the nicker.

Facial Action	Z	p	r	s>n	s<n	s=n
Lower face						
AU12, lip corner puller	n/a	n/a	n/a	n/a	n/a	n/a
AUH13, nostril lift	**-2.35**	**0.019***	**0.34**	**11**	**4**	**14**
AU17, chin raiser	-0.52	0.61	n/a	6	7	16
AU18, lip pucker (count)	**-2.39**	**0.017***	**0.31**	**8**	**1**	**20**
AU18 lip pucker (time)	**-2.43**	**0.015***	**0.32**	**8**	**1**	**20**
Eye region						
AU101, inner brow raise	-0.29	0.77	n/a	14	10	3
AD1, increased eye white	-0.94	0.35	n/a	9	13	6
AU5, lid raiser	**-3.22**	**0.001***	**0.43**	**13**	**0**	**15**
AU47, half blink	-0.60	0.55	n/a	12	16	2
Ears						
EAD103, ear flattener	-0.43	0.67	n/a	12	8	12
EAD104, ear rotator	-0.45	0.66	n/a	15	11	6

hearing the squeal, 19 turned the right (giving their left eyes/ears priority), p=0.001 (two-tailed binomial probability) whereas only 2 turned left. This pattern was not seen in response to the nicker, where horses showed no preference for viewing the speaker with their left eye (*Mdn* = 0 s) compared to their right eye (*Mdn* = 0 s), and an equal number of horses turned their heads right (n=11) as left (n=11), p=1 (two-tailed binomial probability).

Overall, the time spent engaging in approach and avoid behaviours was low (except for some extreme values) making statistical analysis of these behaviours inappropriate. However, more horses displayed approach behaviours to the nicker (n=9) than the

squeal (n=3), and more horses displayed avoidance behaviours to the squeal (n=7) than the nicker (n=5). Horses also spent more time adopting a frozen posture (*Mdn* =7.4 s) in response to the squeal than to the nicker (*Mdn* = 0.87 s), and were more likely to display prolonged looking to the squeal (n=18) than the nicker (n=12), but neither of these comparisons were statistically significant, (frozen: Z=0.55, p=0.58; prolonged longing: McNemar p=0.18). Critically, no horses responded to the calls with either a nicker or a squeal. Four horses vocalised in response to the squeal, and seven horses vocalised in response to the nicker; all vocalisations were whinnies.

Horse spent significantly more time with their ears forward after hearing the nicker (*Mdn*=22.49 s) than the squeal (*Mdn*=21.00 s), Z=-2.24, p=0.03. Horses spent too little time with their ears back to do statistical analysis, but there was a trend towards horses being more likely to hold their ears in a symmetrical position after the nicker (*Mdn*=22.49 s) than the squeal (*Mdn*=21.32 s), Z=1.87, p=0.06. In line with the looking behaviour, horses showed a preference for listening to the squeals with their left ear. In response to the squeal, horses were significantly more likely to have their left ear forward and right ear back (*Mdn*= 0.52 s) than the right ear forward and left ear back (*Mdn*=0 s), Z=-2.26, p=0.02. There was no difference in the time horses spent with their left ear forward/right ear back (*Mdn*=0 s) and right ear forward/left ear back (*Mdn*=0 s), Z=0.42, p=0.68.

Discussion

In our study, hearing a negative affective vocalisation elicited multi-modal mirroring in horses through the spontaneous and rapid production of corresponding affective facial expressions. This was in the absence of any visual input or contextual cues. Preferences for the left eye/ear in response to the negative calls demonstrate that the stimuli were clearly perceived and processed differently, and in line with their presumed affect. Yet there was no strong behavioural reaction to the calls, and so the production of facial expressions in our trials cannot be attributed to an automatic display that was part of a larger behavioural response. Thus, our findings demonstrate the first evidence of audio-motor representation for the transfer of emotional information in a non-human animal.

This specific auditory-motor representation is particularly interesting because when

humans adopt emotional postures and facial expressions they experience the associated emotion (13, 17). This emotional state matching (emotional contagion) is another perception-action mechanism that is critical for understanding the emotions and internal states of others, is thought to help drive social decision-making, and is a pre-cursor to complex social behaviours such as empathy and altruism (18-22). However, whether non-human animals also experience this emotional contagion when performing affective facial mimicry is still unknown.

It is intriguing that there was no mimicry in response to the positive vocalisation (nicker). The production of this call had fewer specific facial actions associated with it, and one of those key actions - the half blink – naturally occurs frequently in horses, potentially introducing an element of noise. However audio-motor representation of affect is also only seen in response to certain vocalisations in humans (albeit positive vocalisations, 6). The existence of mirroring has raised the interesting question of why we do not always imitate what we see (23). Behavioural mirroring may only be a by-product of using auditory-motor representations to represent actions, and potentially the auditory-motor representation was activated more strongly in response to the negative call due to the higher survival implications of recognising a negative situation compared to a positive situation. Similarly, this system may also have preparatory functions for future actions that may be more salient in a negative context than a positive context (1, 5, 24).

To understand complex communication and cognition we need to determine the fundamental mechanisms that underpin behaviour, and the correspondence between sensory and motor codes used to represent inputs (2, 22). Perception-action representations are a physiological mechanism through which this correspondence can be established, and offer a way of representing social information (25). The existence of an emotional audio-motor mirror system in an animal so far removed from the primate lineage suggests that either the evolutionary origins of this mechanism are deep rooted in the last common ancestor of horses and primates (or before), or have evolved convergently in separate social species. Elucidating which of these options are most feasible will have strong implications for how we understand cognition and behaviour.

Acknowledgments

We would like to thank everyone at Glebe Field Equestrian Centre for allowing us access to their horses and to conduct this research on their premises. Leanne Proops and Dan Rubenstein kindly provided recordings of vocalisations that were used as the stimuli. Thanks to Charlotte Lillis and Kate Grounds for their help with data collection. Charlotte also provided some video recordings of horses vocalising, and was the valuable ‘third party’ who selected the specific calls from the video clips to be EquiFACS coded, and provided the trial start and end times for video coding. Kate assisted with video coding of the EquiFACS responses and Verity Bridger assisted with the behavioural video coding. A final acknowledgment must be given to Jamie Ward for his helpful feedback on a previous version of this manuscript. This research was funded by a studentship from the BBSRC and the School of Psychology, University of Sussex, and a JRA award from the University of Sussex.

References

1. Kohler E, Keysers C, Umilta MA, Fogassi L, Gallese V, Rizzolatti G. Hearing sounds, understanding actions: action representation in mirror neurons. Science. 2002;297(5582):846-8.
2. Prather JF, Peters S, Nowicki S, Mooney R. Precise auditory-vocal mirroring in neurons for learned vocal communication. Nature. 2008;451(7176):305-10.
3. Mukamel R, Ekstrom AD, Kaplan J, Iacoboni M, Fried I. Single-neuron responses in humans during execution and observation of actions. Curr Biol. 2010;20(8):750-6.
4. Keysers C, Kohler E, Umiltá MA, Nanetti L, Fogassi L, Gallese V. Audiovisual mirror neurons and action recognition. Exp Brain Res. 2003;153(4):628-36.
5. Aglioti SM, Pazzaglia M. Representing actions through their sound. Exp Brain Res. 2010;206(2):141-51.
6. Warren JE, Sauter DA, Eisner F, Wiland J, Dresner MA, Wise RJ, et al. Positive emotions preferentially engage an auditory-motor "mirror" system. The Journal of neuroscience. 2006;26(50):13067-75.
7. Winston J, O'Doherty J, Dolan R. Common and distinct neural responses during direct and incidental processing of multiple facial emotions. Neuroimage. 2003;20(1):84-97.
8. Van der Gaag C, Minderaa RB, Keysers C. Facial expressions: what the mirror neuron system can and cannot tell us. Soc Neurosci. 2007;2(3-4):179-222.
9. Hennenlotter A, Schroeder U, Erhard P, Castrop F, Haslinger B, Stoecker D, et al. A common neural basis for receptive and expressive communication of pleasant facial affect. Neuroimage. 2005;26(2):581-91.
10. Banissy MJ, Sauter DA, Ward J, Warren JE, Walsh V, Scott SK. Suppressing sensorimotor activity modulates the discrimination of auditory emotions but not speaker identity. The Journal of Neuroscience. 2010;30(41):13552-7.
11. Adolphs R, Damasio H, Tranel D, Cooper G, Damasio AR. A role for somatosensory cortices in the visual recognition of emotion as revealed by three-dimensional lesion mapping. The Journal of Neuroscience. 2000;20(7):2683-90.
12. Pitcher D, Garrido Lc, Walsh V, Duchaine BC. Transcranial magnetic stimulation disrupts the perception and embodiment of facial expressions. The Journal of Neuroscience. 2008;28(36):8929-33.
13. Niedenthal PM. Embodying Emotion. Science. 2007;316(5827):1002-5.
14. Harrison NA, Morgan R, Critchley HD. From facial mimicry to emotional empathy: a role for norepinephrine? Soc Neurosci. 2010;5(4):393-400.
15. Ekman P, Friesen WV, Hager JC. Facial Action Coding System Investigator's Guide. Salt Lake City, USA: Research Nexus; 2002.
16. Wathan J, Burrows A, Waller BM, McComb K. EquiFACS: the Equine Facial Action Coding System. PLOS One 2015;in press.
17. Hess U, Kappas A, McHugo GJ, Lanzetta JT, Kleck RE. The facilitative effect of facial expression on the self-generation of emotion. International Journal of Psychophysiology. 1992;12(3):251-65.
18. de Waal FB. Putting the altruism back into altruism: the evolution of empathy. Annual Review of Psychology. 2008;59:279-300.
19. Preston SD, De Waal F. Empathy: Its ultimate and proximate bases. Behavioral and Brain Sciences. 2002;25(01):1-20.

20. Lakin J, Jefferis V, Cheng C, Chartrand T. The Chameleon Effect as Social Glue: Evidence for the Evolutionary Significance of Nonconscious Mimicry. J Nonverbal Behav. 2003;27(3):145-62.
21. Wathan J, Proops L, Grounds K, McComb K. Functional use of facial expressions in a non-primate: horses discriminate between emotional conspecific facial expressions. Under review. 2015.
22. de Waal FB, Ferrari PF. Towards a bottom-up perspective on animal and human cognition. Trends Cogn Sci. 2010;14(5):201-7.
23. Keysers C, Gazzola V. Social neuroscience: mirror neurons recorded in humans. Curr Biol. 2010;20(8):R353-4.
24. Leslie KR, Johnson-Frey SH, Grafton ST. Functional imaging of face and hand imitation: towards a motor theory of empathy. Neuroimage. 2004;21(2):601-7.
25. Gazzola V, Aziz-Zadeh L, Keysers C. Empathy and the somatotopic auditory mirror system in humans. Curr Biol. 2006;16(18):1824-9.

Article III

The Eyes and Ears are Visual Indicators of Attention in Domestic Horses

Wathan, J. & McComb, K. (2014). *Current Biology, 24*(15), R677-R679.

Summary

Sensitivity to the attentional states of others has adaptive advantages [1] and in social animals attending to others is important for predator detection, as well as a pre-requisite for normal social functioning and more complex socio-cognitive abilities [2]. Despite widespread interest in how social species perceive attention in others, studies of non-human animals have been inconclusive about the detailed cues involved [3]. Previous work has focused on head and eye direction, overlooking the fact that many mammals have obvious and mobile ears that could act as a visual cue to attention. Here we report that horses use the head orientation of a conspecific to locate food, but that this ability is disrupted when parts of the face (the eyes and ears) are covered up with naturalistic masks. The ability to correctly judge attention also interacted with the identity of the model horse, suggesting that individual differences in facial features may influence the salience of cues. Our results indicate that a combination of head orientation with facial expression, specifically involving both the eyes and ears, is necessary for communicating social attention. These findings emphasise that in order to understand how attention is communicated in non-human animals, it is essential to consider a broad range of cues.

Main Text

Studies using naturalistic gaze following paradigms have indicated that a wide range of animals, from crows to chimpanzees, follow the attention of conspecifics [2]. However, gaze is often used as a general term encompassing head orientation, eye direction and any other potential indicators. Consequently, it is difficult to establish exactly what cues are informative, and previous experimental work exploring this has focused on cues that humans use (head orientation and eye gaze) [3, 4], potentially overlooking a wealth of other available information. Animals with a different facial morphology – particularly those with large, mobile ears – may have other means of signalling.

Horses are a prey animal with advanced social relationships [5-7], and within the domestic environment they often have parts of their faces covered by riding equipment or masks used for protection from flies. We used these masks, presented within photographic stimuli, to investigate whether horses were responsive to the attentional cues of another horse, and if so what areas of the face were important in providing information (see Figure 23A; Supplemental Information and Figure 24).

In our experiment, horses were clearly sensitive to the attentional state of a conspecific and this influenced their decision about where to feed. When subjects viewed the unoccluded image of another horse looking at one of two buckets containing food, they were more likely to feed from the bucket congruent with the model (n = 24, K = 18, P = 0.02, see Figure 23B). However, when either the eyes or ears were covered the choices of the participants dropped to chance levels (eyes: n = 24, K = 14, P = 0.54, ears: n = 24, K = 12, P = 1) suggesting these were both key areas informing the participants' decisions.

The cues available (whole head visible, eyes covered, or ears covered) also influenced time spent looking at the photographs, $F(2,62) = 3.62$, P=0.03; Supplemental Information. Planned comparisons revealed that horses looked for significantly longer when all the information was visible, compared to when the ears or the eyes were covered (P<0.01). However, there was no difference in looking time when the ears were covered compared to when the eyes were covered, nor was looking time influenced by the identity of the model horse.

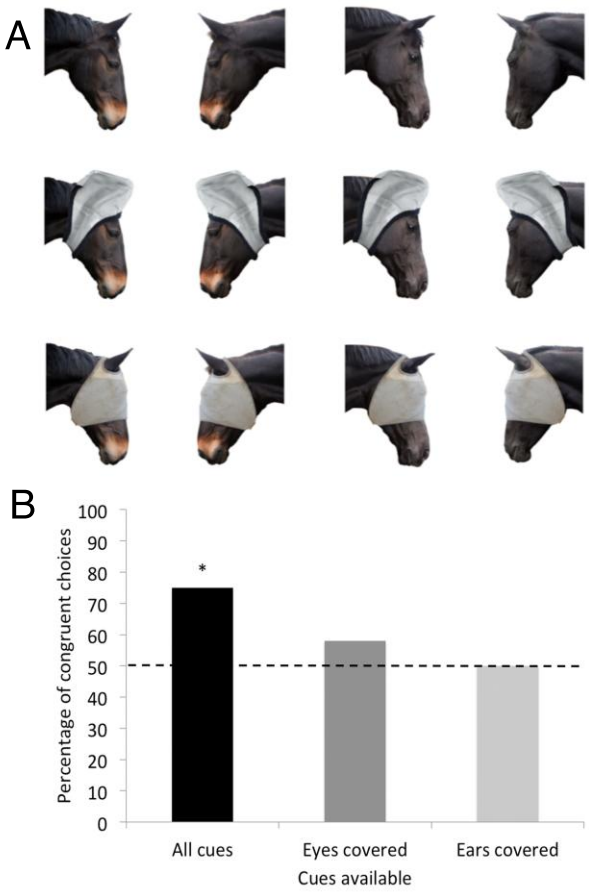


Figure 23. Illustration of stimuli and main results. A. Photographic stimuli including manipulations that incorporate fly masks to cover key internal features of the face. The photographs were reproduced at life size and used as the model in an object choice task to establish whether horses could use the head orientation and facial expression of a conspecific to locate hidden food. Both model horses are shown here in the three experimental conditions: all cues visible; ears covered; eyes covered. WG is the horse on the left of the image, MC is the horse on the right. B. Percentage of horses choosing the congruent bucket for each condition. * $P < 0.05$ (binomial probabilities, two tailed).

However, more subtle effects were also apparent. Cues available, age, sex, testing centre, model horse viewed, and stimuli direction were entered as predictors in a logistic regression with feeding choice as the response variable (0 = choice incongruent with model, 1= choice congruent with model; Supplemental Information and Table 6). This revealed a significant interaction, whereby sensitivity to the cues available differed according to the model horse viewed. Covering the eyes (Figure 23A) had less influence

on subjects that viewed MC than subjects who viewed WG (see also Supplemental Information), potentially indicating that differences in the facial features or expression of the two models affected the salience of cues, and highlighting an interesting area for future research.

We also conducted additional presentations of single images as controls to verify that covering key parts of the face did not impede subjects' recognition of the stimuli as depicting a horse. When subjects were allowed to view our horse stimuli at close range they showed similar reactions to all three conditions (all cues visible, eyes covered, ears covered), which were significantly different from their responses to appropriately matched control stimuli (phase-scrambled counterparts of the originals; Supplemental Information, Figure 25). Furthermore, subjects were more likely to approach the original stimuli and more likely to avoid the controls, as would be predicted if they were responding to photographs of horses versus novel objects (see details in Supplemental Information). We also took precautions to avoid the possibility of a 'Clever Hans Effect' occurring through incidental cueing by the experimenter. In particular, the experimenter was unfamiliar to the horse, kept ignorant of which stimuli would be presented and, crucially, after the release point (when the choice was made) they stood facing away from the horse, so could not see the horse's choice or provide any feedback (full details in Supplemental Information).

Our results provide the first evidence from an animal with laterally placed eyes that cues from this area convey important information. Eye gaze is difficult to isolate in animals with eyes positioned at an oblique angle, and it had been suggested that non-primates cannot use eye gaze independently of head orientation [2, 4]. However, we demonstrate that the eyes do carry information, even when laterally placed in an animal far removed from the primate lineage. Horses, along with other ungulates, have a white sclera that is visible in various situations [6]. This plus other cues, such as dilation of the pupil and movement of the facial muscles surrounding the eye, could be informative of attentional state, as they are in humans [8].

Most significantly, our results demonstrate that animals with large, mobile ears can use these as a visual cue to attention. While anecdotal accounts of this exist in the literature (e.g. [6]) the potential role of the ears in signalling has been overlooked in previous

experiments. In animals that have evolved a differently shaped face it is important to consider cues that humans do not have, and novel paradigms that incorporate these will be crucial in developing a full understanding of attentional mechanisms across species.

Supplemental Information

Supplemental experimental procedures

Study animals

A total of 82 horses were tested and of these 72 were included in the final analysis. Reasons for exclusion were: methodological error (2); approached the set up at an oblique angle (5); horses chose not to participate (3). Of the 72 horses included in the final analysis, 52 horses were geldings and 20 mares. Ages ranged from 3 – 30 years (M =14.97, SD = 5.90). Subjects had no known eyesight problems. Horses were from several yards in the South-East and North-West of England and were housed under various conditions. This ranged from horses that were kept outside all year round to horses that were stabled 24 hours a day during winter and turned out in the summer. Some horses lived with between 0 and 6 regular companions, whereas others were turned out with groups that varied in size and composition. All horses had experience of a field companion wearing fly masks; however, only 7 horses had personal experience of actually wearing the masks. All testing was completed in the indoor arena on the yard where the participant resided.

Training trials

All horses participated in one training trial. A bucket containing 20g of concentrated hard feed was placed in front of a plastic block at the edge of an indoor arena (Figure 24B). Horses were led into the arena and walked in a figure of eight before being turned towards the training set up. Horses were released at a point (R) 5m away from the apparatus to approach the bucket and receive the food reward (Figure 24A). If the horses did not approach then the experimenter encouraged them. Once the horse had eaten it was caught and lead away while the experimental apparatus was set up. If the horse did not choose to approach and eat from the bucket it did not move forward to the test trial.

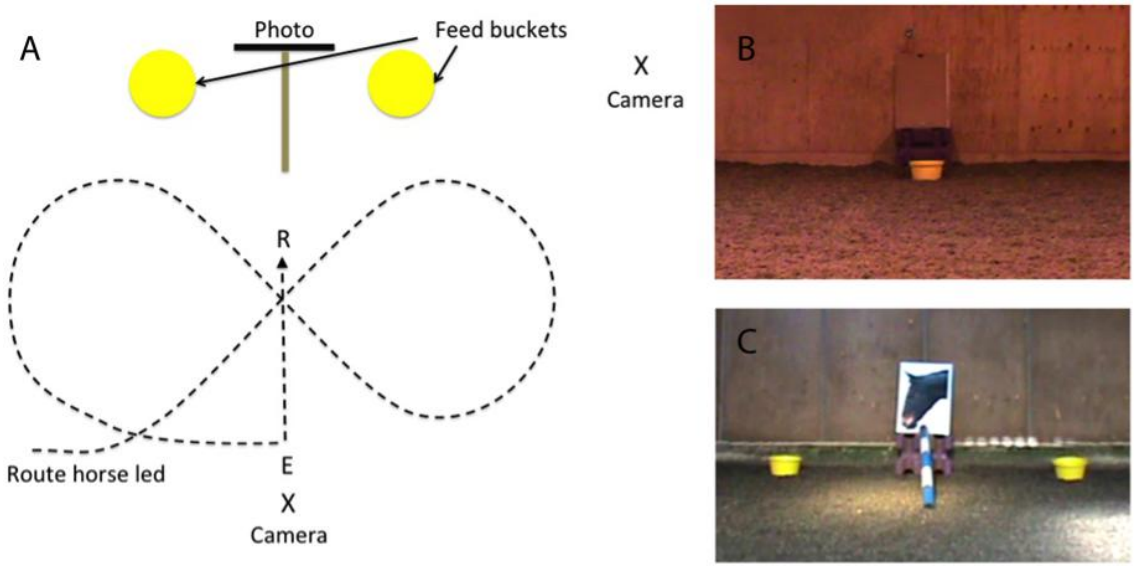


Figure 24. The experimental set up. A. Diagram showing the testing area and route taken to the release point (R). All trials were conducted in an indoor area on the yard where the participant resided. Horses participated in one training trial (B) in which a bucket containing 20g of hard feed was placed against the wall. Horses were brought into the area and led in a figure of eight before being released (at point R) to approach the bucket and eat the food. If horses were happy to approach the bucket they then participated in one test trial (C) where a photographic stimulus was attached to the wall above a dividing pole raised at one end on a plastic block, with a bucket containing 20g hard food on either side (Stimulus shown in picture is WG, whole head visible). Horses were again walked in a figure of eight before being released at point R.

Test trials

Once the horse had been lead away, a second person set up the test trial so the experimenter leading the horse was blind to the sequence of presentation. The photographic stimulus was attached to a wall, and a wooden pole was placed perpendicular to the wall, with one end raised up on a block directly under the photograph (Figure 24C). The pole was to encourage the horses to make an unambiguous choice. One bucket was placed 150cm either side of the dividing pole. Both buckets contained an identical food reward (20g concentrated food) to remove the possibility of horses using odour to locate the food. Once the test apparatus was set up the horse was brought back and led in another figure of eight before turning in and being released at point R, 5m away from the photograph (Figure 24A). Upon release the

experimenter leading the horse walked away and stood directly behind the horse facing away from the experimental set up (point E). This was done to make the experimenter blind to the horses' responses, and to remove the opportunity for the horse to pick up on incidental cues (the Clever Hans Effect). In addition to this the experimenter was blind to the sequence of presentation, was instructed to look at the floor during leading, and wore a peaked cap to help obscure the view of the photograph. Horses were given two minutes to approach the set up and make a choice. If they did not approach within this time (or wandered away from the trial) then they were caught, walked in another figure of eight, and released again. Horses that did not make a choice after 3 releases were counted as non-responders.

Stimuli

Photographs were taken of horses looking at a bucket of food on the floor 150cm away. Two horses were used as the models; a 15 year old bay Irish draft x thoroughbred gelding (WG) and a 10 year old black Dutch sports horse mare (MC). The models were unfamiliar to the participants, to avoid potential confounding effects of dominance. Photographs were taken with a Canon 400D DSLR camera. Two photographs of each horse were selected (one looking left and one looking right) to create the final stimuli set. The images were then extracted, placed onto a uniform white background, and auto adjusted for levels and brightness in Adobe Photoshop. Further stimuli were created by adding eye and ear masks with Photoshop to obscure the view of the eyes or ears. These masks are commonly used for horses that are sensitive to flies, and the nature of the fabric means that although horses can see out of them, the external view of either the eyes or ears is obscured. This created six stimuli of each model horse (whole head left and right; eyes covered left and right; ears covered left and right – see main text for a visual representation). The stimuli were enlarged to A1 (841 x 594 mm) and printed.

Stimuli Validation

Sheep, a domestic ungulate with similar vision to horses, show remarkable abilities for recognising the faces of other sheep in photographic stimuli (for an example see [S1]). Consequently it was expected that horses would also be able to recognise the photographic stimuli developed for this experiment as depicting a horse, even when key parts of the face were covered. If the horses did not recognise the photographs, we

would expect them to react to the stimuli as they would to any other comparable novel object.

To explore this, we compared reactions to the stimuli to reactions to an appropriately matched novel object in a separate experiment. The novel objects used were matched, phase-scrambled images of the stimuli. Phase-scrambled images contain the same physical energy properties as the original image, but are rearranged so none of the original facial features are distinguishable; making them an ideal control for recognition tasks (personal communication, Graham Hole; see Figure 25 and [S2] for an example). The phase-scrambled images were generated in Matlab R2010a.

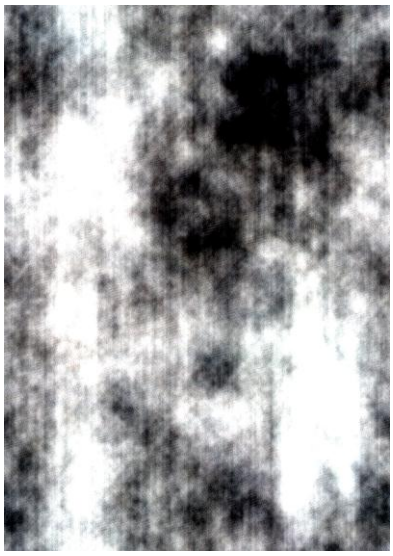


Figure 25. An example of the phase scrambled images. This image is a phase scrambled example of Horse 1, eyes covered, facing left.

24 horses (aged from 11–21 years, *M* = 16.95, *SD* = 2.97; 20 geldings, 4 mares) participated in a mixed design. Each horse saw one standard version of a stimulus and the corresponding scrambled version in separate presentations. Time between presentations was at least 7 days (*M*=23.63, *SD*=19.27) and the first presentation varied equally between the standard and scrambled stimuli. Stimuli were presented for a total of 30 seconds: An experimenter initially held the stimuli up at a point 60cm from the horse's nose for 10 seconds, then moved it forward approximately 10cm and held it there for 10 seconds, before moving it back to the starting position for a final 10

seconds. A second experimenter held the horse on a loose rope, facing the rear of the horse so she could not see the procedure and incidentally cue the horse. Trials were video recorded, and time spent looking at the stimuli, time spent avoiding the stimuli, and time spent approaching the stimuli were measured.

One-way ANOVAs demonstrated that the horses appraised the three standard stimuli in a similar way. There was no significant difference in avoidance behaviour, approach behaviour, or looking behaviour across the three conditions of standard stimuli, $p>0.05$ for all comparisons; as these horse stimuli did not elicit different responses, the following comparisons reflect responses to the three conditions of standard stimuli - all cues visible, eyes covered, and ears covered - combined in comparison with the control stimuli. Importantly, horses reacted significantly differently to the standard stimuli than to the scrambled control stimuli. Horses were significantly more likely to approach the horse stimuli (M=7.60, SEM= 1.49) than the control stimuli (M=3.83, SEM=1.63), $t(23)=2.45$, $p=0.02$. In addition, horses were significantly more likely to avoid the scrambled stimuli (M=13.67, SEM=2.25) than the horse stimuli (M=0.82, SEM=0.33), $t(23)=5.98$, $p<0.001$. There was no difference in looking time between the horse stimuli (M=17.78, SEM=1.42) and the scrambled stimuli (M=14.84, SEM=1.57), $t(23)=1.33$, $p>0.05$. From these comparisons we concluded that subjects were able to recognise the photographic stimuli as depicting horses, even when the masks covered some of the internal features of the face.

Ethical statement

The study was reviewed and approved by the University of Sussex Ethical Review Committee. The Association for the Study of Animal Behaviour Guidelines for the Treatment of Animals in Behavioural Research and Teaching were adhered to. Owners/care takers of the horse gave consent prior to participation. Horses were not food deprived and remained in their familiar environment during participation in the study.

Video analysis

All videos were analysed frame by frame using Sportscode Gamebreaker 7.5.5. Coding was performed by JW, with 15% (11/72) of videos for the main experiment and 17% (8/24) of videos for the validation trials coded by a second observer. Cronbach's alpha

revealed good to excellent levels of agreement for the measures of interest: bucket chosen to feed from (1); latency to approach (1); time looking at photograph (0.82); time avoiding the stimuli (0.87); time approaching the stimuli (0.87). The video analysis for the main trials was performed blind to the experimental condition.

Statistical analysis

The main behaviour studied was which bucket the responding horse chose to feed from, and whether the bucket was congruent or incongruent with the bucket the model horse was looking at. Accuracy was assessed using two-tailed binomial tests.

To assess any potential influence of age and sex of the horse, which centre the horse was tested at, the model horse, and the direction the stimuli pointed, these variables were entered along with condition/cues visible as predictors in a logistic regression. Choice was used as the binary response variable (0 = choice incongruent with model, 1= choice congruent with model). Whether inclusion or exclusion of parameters in the model significantly improved the fit was tested by comparing the difference between the deviance values of two models, which are distributed approximately as chi-squared (χ^2) with degrees of freedom (df) equivalents to the difference in the number of parameters fitted in each model [S3].

The potential influence of cues visible, model horse seen, and congruent choice on the latency to approach and time looking at the photograph (measured in seconds) were analysed using a 3-way independent ANOVA. Planned comparisons were used to follow up significant results.

Supplemental results

Table 6. Results of a linear regression analysis to determine the parameters affecting accuracy (0=incongruent choice, 1=congruent choice).

Variable	**Deviance (χ^2)**	***df***	***P***
Cues visible	4.38	2	0.11
Model	0.55	1	0.46
Cues visible*Model	7.03	2	0.03*
Stimuli direction	0.62	1	0.86
Centre	2.54	6	0.85
Age	0.22	1	0.64
Sex	0.33	1	0.57
Sex*Age	1.20	2	0.55

Interaction between cues visible and model

The model that best fitted the data contained cues visible, model horse viewed, and the interaction between these variables (Table 6). This model fitted the data significantly better than the null hypothesis, χ^2 (6) = 14.70, *P*=0.02. The interaction in this model reveals that sensitivity of accuracy to cues visible differed according to the model horse subjects were looking at. Fisher's Exact Tests demonstrated that when viewing MC subjects had a higher accuracy rate when the eyes were covered (ears visible) than when viewing WG (MC 83% vs WG 33% accuracy, P=0.04). However, there was no difference when the ears were covered (MC 42% vs WG 58% accuracy, P=0.68) or when all cues were visible (MC 67% vs WG 83% accuracy, P=0.64).

Time spent looking at the photograph and latency to approach a bucket

Horses looked at the photograph for longest when the whole head was uncovered (*M*=3.21s, *SEM*=0.91) and looked for less time when the eyes and ears were covered (eyes: *M*=1.52s, *SEM*=0.48; ears: *M*=1.14s, *SEM*=0.21; see main text for statistical comparison). None of the variables of interest (cues visible, model horse seen, and congruency of choice) influenced latency to approach a bucket (*P*>0.05 for all comparisons).

Acknowledgements

We would like to thank the horses and owners at Bowler's Riding School, Albourne Equestrian Centre, Plumpton Agricultural College, Hope in the Valley Riding for the Disabled Group, Ditchling Common Stud, and Westfield Equestrian Centre for their participation in this research. Thanks to Andy Bamford, Becky Spooner, Amy Smith, Chris Wathan, and Vicky Ratcliffe for their help with data collection. Also thanks to Becky for doing the second video coding, Andy for invaluable advice on the statistical analysis, and Graham Hole for his help and advice developing the control stimuli for the stimuli validation tests. Finally, thanks to Leanne Proops, Graeme Shannon, and the three reviewers for their support and helpful comments on the manuscript. This work was funded by a PhD studentship awarded to JW (supervised by KM) from the BBSRC and the University of Sussex.

References

Main text

1. Goodwin, D. (2002). Horse behaviour: evolution, domestication, and feralisation. In The Welfare of Horses, N. Waran, ed. (Dordrecht: Kluwer), pp. 19 - 44.
2. Emery, N.J. (2000). The eyes have it: the neuroethology, function and evolution of social gaze. Neuroscience & Biobehavioral Reviews *24*, 581-604.
3. Rosati, A.G., and Hare, B. (2009). Looking past the model species: diversity in gaze-following skills across primates. Curr Opin Neurobiol *19*, 45-51.
4. Kaminski, J., Riedel, J., Call, J., and Tomasello, M. (2005). Domestic goats, Capra hircus, follow gaze direction and use social cues in an object choice task. Animal Behaviour *69*, 11-18.
5. Proops, L., McComb, K., and Reby, D. (2009). Cross-modal individual recognition in domestic horses (Equus caballus). Proc Natl Acad Sci U S A *106*, 947-951.
6. Waring, G.H. (2003). Horse Behavior, (Noyes Publications/William Andrew Pub.).
7. Cozzi, A., Sighieri, C., Gazzano, A., Nicol, C.J., and Baragli, P. (2010). Post-conflict friendly reunion in a permanent group of horses (Equus caballus). Behav Processes *85*, 185-190.
8. Wierda, S.M., van Rijn, H., Taatgen, N.A., and Martens, S. (2012). Pupil dilation deconvolution reveals the dynamics of attention at high temporal resolution. Proc Natl Acad Sci U S A *109*, 8456-8460.

Supplemental References

S1. Tate AJ, Fischer H, Leigh AE, Kendrick KM (2006) Behavioural and neurophysiological evidence for face identity and face emotion processing in animals. Philosophical transactions of the Royal Society of London Series B, Biological sciences *361*, 2155-2172.

S2. Bukowski H, Dricot L, Hanseeuw B, Rossion B (2013) Cerebral lateralization of face-sensitive areas in left-handers: Only the FFA does not get it right. Cortex *49*, 2583-2589.

S3. Dobson AJ (2010) An Introduction to Generalized Linear Models, Second Edition: Taylor & Francis.

Article IV

Functional use of Facial Expressions in a Non-Primate: Horses Discriminate Between Differing Emotions.

Wathan, J., Proops, L. Grounds, K., & McComb K.

In the style of Proceedings of the Royal Society B: Biological Sciences.

Abstract

In humans, facial expressions are a rich source of social information. However, the extent to which this is true in non-human animals remains largely unknown. Here we report that horses discriminate between emotional facial expressions of conspecifics and use this to inform their social behaviour. Horses were more likely to approach photographic stimuli of an unfamiliar conspecific displaying a positive facial expression and more likely to avoid stimuli displaying a negative expression. Moreover, subjects' heart rates (a key indicator of internal state) rose significantly higher and took significantly longer to return to resting baseline in response to viewing the negative facial expression compared to the positive expression. Our results demonstrate that animals far-removed from the primate lineage have the ability to use facial expressions as a means of gaining social information, and suggest that this ability is either an evolutionarily conserved trait or has evolved under common selective pressures in these diverse taxa.

Introduction

Faces are a source of potentially valuable 'public information' that can be freely obtained, whether given purposefully or not by the signaller [1]. Faces can have features characteristic of age, sex and identity, and this information is processed rapidly and often subconsciously [2]. Facial expressions can represent another's internal states (e.g. pain) and consequently accurate perception and recognition of facial expressions has the potential to make subsequent behaviour more predictable, facilitating social interactions and bonding [3]. This fundamental role at the core of social interaction is particularly important for group living species, where the management of relationships and group cohesion is essential for the maintenance of complex social networks [4-8]. However, while many non-human animals demonstrate distinctive facial expressions, some of which clearly resemble those seen and recognised cross culturally in humans [6], the functional significance of animal facial expressions has received surprisingly little attention.

Darwin established early on that a wide variety of animals have the capacity for meaningful facial displays [9], but few studies went on to directly examine these or uncover their meaning, function, and evolutionary history. Despite this, it is repeatedly claimed that primates use their facial expressions in a unique way compared to non-primate orders, with other animals only having a limited capacity for facial expression [10, 11-15]. However, because research investigating the production of facial expressions has been biased heavily towards primates, the true ability of other species remains largely unknown [3, 13]. Recent behavioural and anatomical research investigating the capacity for and production of facial expressions within the primates has revealed interesting patterns, which suggest that further investigation across more taxa is necessary. For example, the facial displays and muscles of some primates, such as *Otolemur* (galagos or bushbabies), have a more complex facial musculature network than previously reported, with *Otolemur* displaying the same number of facial muscles as black lemurs (although arranged in a different structure), and only slightly less than rhesus macaques, hylobatids, and chimpanzees (17 muscles versus 19-20) [16-20]. Furthermore, although similar in arrangement, the facial muscles of hylobatids are more gracile than previously reported, being less developed than those of rhesus macaques [16, 18, 19]. In line with this, new research systematically documenting the facial

muscles and expressions of the domestic horse, dog, and cat has demonstrated extensive capacity and a surprising amount of similarity with humans and other primates [21-26]. A range of different facial expressions have also been documented in plains zebra, fur seals, and walruses [3, 27]. Clearly, animals other than the primates are able to produce complex facial expressions, and investigating the continuity between species, and how this relates to social and ecological variables, could reveal a much broader evolutionary context.

Experimental work systematically examining what information receivers extract from signals and how this may be functionally relevant has been particularly neglected in comparison to work on the production of facial expressions [3, 28]. Captive chimpanzees and crested macaques can spontaneously match corresponding emotional facial expressions occurring in different individuals, and chimpanzees can also match a conspecific facial expression to a context of similar valence (e.g. a scream face to scenes of chimps being injected during a veterinary procedure) [6, 29]. These results indicate that chimpanzees infer some, seemingly categorical, meanings from the expressions, and are amongst the few studies in non-humans to explicitly address questions about the wider perception of facial expressions [3]. Domestic dogs show differing gaze biases when viewing photographs of conspecific facial expressions [28], and can, after training, learn to discriminate between photographs of human facial expressions [30, 31]. It has also been reported that sheep can discriminate between emotional faces of conspecifics and humans, but the experimental details and results of this are not explicitly provided [14]. Interestingly, a recent study showed that rats did not seem to be able to use facial expressions alone to discriminate conspecifics displaying pain from neutral rats, despite the previous identification of a rat pain face [32, 33]. Crucially, no study has yet demonstrated the ability to spontaneously perceive and discriminate conspecific facial expressions in a functionally relevant way in any non-primate species. Here we use naturalistic approach/avoidance paradigms alongside physiological (heart rate) measurement, to investigate the perception of conspecific facial expressions in a highly social non-primate: the domestic horse.

Horses are gregarious creatures, forming strong social bonds and processing information on social companions in a cognitively complex way [34, 35]. In line with this, horses exhibit sophisticated patterns of social interaction, such as post-conflict

affiliation, that are considered characteristic of advanced social cognition [36, 37]. In the wild, horses live in large societies comprised of several sub-groups that share space and resources in overlapping home ranges, and to which membership stays relatively stable over time [38]. Thus horses regularly come into contact with many other conspecifics and inter-band dominance indicates that within the larger herd established social relationships exist [38]. Consequently, horses show some degree of fission-fusion dynamics; the same complex social organisation that is seen in humans, bonobos, chimpanzees, and macaques, as well as elephants, spotted hyenas and many cetaceans [8]. Group life in these societies is determined by long-term social relationships that must be maintained, suggesting effective communication would be advantageous. Horses display a wide range of facial expressions and have been shown to gain information about the attention of conspecifics from detailed cues in the face [26, 39, 40].

We conducted two experiments to systematically investigate how horses responded to differing emotional facial expressions of conspecifics. First, we used an approach/avoid paradigm to observe the spontaneous behaviour of horses when they were presented with a paired choice of two life-sized photographs of the face of an unfamiliar conspecific displaying different expressions. In giving the horses the chance to interact freely with the photographs, we could gain insights into how the horses perceived the facial expressions of the model and how the information they gained influenced their social behaviour. The expressions used were captured opportunistically, rather than posed, prototypical expressions, and included a negative (aggressive) expression, a neutral expression, and an expression of positive anticipation. Secondly, we further explored the behavioural and physiological responses to the positive and negative stimuli in an additional experiment where horses viewed the positive and the negative stimuli in single presentations while their behaviour and heart rate was recorded. Physiological measures, such as heart rate, are a well-established method of measuring emotional states [41, 42], and so this second experiment provided insights into the internal states of the horses when viewing the emotional facial expressions.

Methods

Study animals

In experiment 1, 70 horses were recruited from stables in Merseyside, Sussex, and Surrey, UK. Twenty-two of these horses chose not to participate in the experiment (i.e. did not look at or approach the experimental set up and walked away immediately upon release) and so could not be included in the analysis. An additional ten horses were tested but excluded because of a side bias at one center (9/10 horses went left, towards the entrance to the stable block; two tailed binomial probability, P=.02). This left 48 horses in the final analysis: 29 gelded males and 19 mares (3–32 years, M=15.04, SD=6.03).

In experiment 2, 38 horses were recruited from stables in Sussex, UK. Three horses could not complete their second trial (two horses were sold, one horse died) and a further two horses were removed from the data set because of disturbances during a trial, leaving 33 horses in the behavioural analysis (14 mares, 19 gelded males; 5–27 years, M=16.81, SD=4.34). For heart rate analysis, data for one horse were lost due to equipment failure and five horses were removed due to a high proportion of corrections in one of the heart rate recordings (>5%) [43] leaving 27 horses in the final dataset (12 mares, 16 gelded males; 5–27 years, M=16.82, SD=4.64).

Stimuli

High quality photographs were taken of two horses (one adult female, age 10 years; one adult male, age unknown) in different contexts: a ‘positive’ situation, where horses were anticipating the delivery of a high value food reward, a ‘neutral’ situation, where horses were relaxed, and a ‘negative’ situation, where one horse was having her underside touched and the other had been left in the stable while his stable-mates were taken out to the field (aversive situations for these horses, Figure 26). These expressions were characterized using the Equine Facial Action Coding System (EquiFACS [26]) showing quantitative differences between contexts (Supplementary Material). Photographs were taken with a Canon 350D DSLR camera. Three photographs of each horse (one from each context) were selected to create the final stimuli set. The images were then extracted, placed onto a white background, and auto adjusted for levels and

brightness in Adobe Photoshop. The stimuli were enlarged to A1 (841x594 mm), printed and laminated.

Figure 26. Photographic stimuli. Facial expressions shown from left to right are positive, neutral, and negative. Horse 1 is displayed at the top of the image and horse 2 at the bottom of the image. See Supplementary Material for the EquiFACS codes describing the expressions.

Ethical statement

This work complies with the Association for the Study of Animal Behaviour guidelines for the use of animals in research and received approval from the Ethical Review Committee at the University of Sussex. Owners/carers of the horses gave consent prior to participation. Horses were not food deprived and remained in their familiar environment during participation in the study.

Experimental procedure: experiment 1, paired choice

Horses were exposed to two photographs of the same horse depicting different facial expressions in an approach-avoidance paradigm. Each horse only participated in one

test trial and was presented with one pairwise comparison in this trial (pairwise comparisons were: positive/negative, neutral/negative, positive/neutral; within a trial both photographs displayed the same model horse; however, the two models displayed in Figure 26 were displayed equally across trials). Each emotion was presented on the left and the right of the experimental set up an equal number of times.

See Figure 27 A for a diagrammatic representation of the experimental set up. In each trial the two relevant stimuli were attached to the wall 1.5m apart at the inner edges and 0.4m from the floor at the bottom edge. Jump poles were placed 1m away from the outer edge of the photographs, perpendicular to the wall. These were raised on a plastic block at one end and fanned out by 0.5m at the other end to create a definable testing area. Once the apparatus was set up, a second experimenter brought the horse into the arena and led it in a figure of eight, before turning in and being released at a point, R, 3m away from the photographs. Upon release the experimenter leading the horse walked away and stood directly behind the horse facing away from the experimental set up, (point E) so she could not see the horse's behaviour or provide any incidental cues.

Upon release horses were allowed to enter the testing area and interact freely with the photographs. The end of the trial was defined as when the horse chose to leave the testing area, up to a point 120s from release; if the horses were still in the testing area at this time they were caught and the trial was ended unless they were still actively investigating the photograph, in which case they were allowed to finish their investigations before the end of the trial. If upon release horses immediately left the test area they were caught, walked in another figure of eight, and released again. Horses that did not approach the set up after 3 releases were counted as non-responders. See Video 51 for an example of a trial. Trials were video recorded on a Canon XM2 and Sony DCR-SR58E handycam or Panasonic HC-V720 and X920 handycams.

Experimental procedure: experiment 2, single presentations

In this experiment each horse was presented with one positive photograph and one negative photograph (of the same model horse) in separate presentations. Time between presentations was 61-167 days (M=81.18, SD=23.17) and presentation order was counterbalanced. Heart rate was monitored for 5 minutes prior to the presentation to

gain an average resting baseline for the horse, for the duration of the presentation, and then for 5 minutes immediately post-test to monitor recovery rate.

See Figure 27 B for a diagrammatic representation of the experimental set up. Stimuli were presented for a total of 30 seconds: an experimenter initially held the stimulus up at a point 1m from the horse's nose for 10 seconds, then moved it forward 0.1m and held it there for 10 seconds, before moving it back to the starting position for a final 10 seconds. The positions for the board to be presented in were marked by lines on the stable floor, to ensure accurate presentation. A second experimenter held the horse on a 1.5m long rope and allowed the horse to move freely within the range of the rope. The experimenter holding the horse stood approximately at the horse's shoulder, facing the rear of the horse so she could not see the stimulus and incidentally cue the horse. The stimuli were presented so that the top of the picture was level with the horse's withers, and the experimenter conducting the presentation kept their head hidden behind the stimulus. A third person arranged the presentation to ensure both experimenters were blind to the stimulus being presented, and therefore any potential 'Clever Hans effect' could be discounted. See Video 52 for an example trial.

Trials were video recorded on Panasonic HC-V720 and X920 handycams with a wide view lens attachment. Heart rate was recorded using a Polar Equine RS800CX heart rate monitor (see Supplementary Material for details of heart rate data processing). An experimenter remained with the horse during the pre and post-test recording time, but did not interact with the horse except to prevent excessive movement.

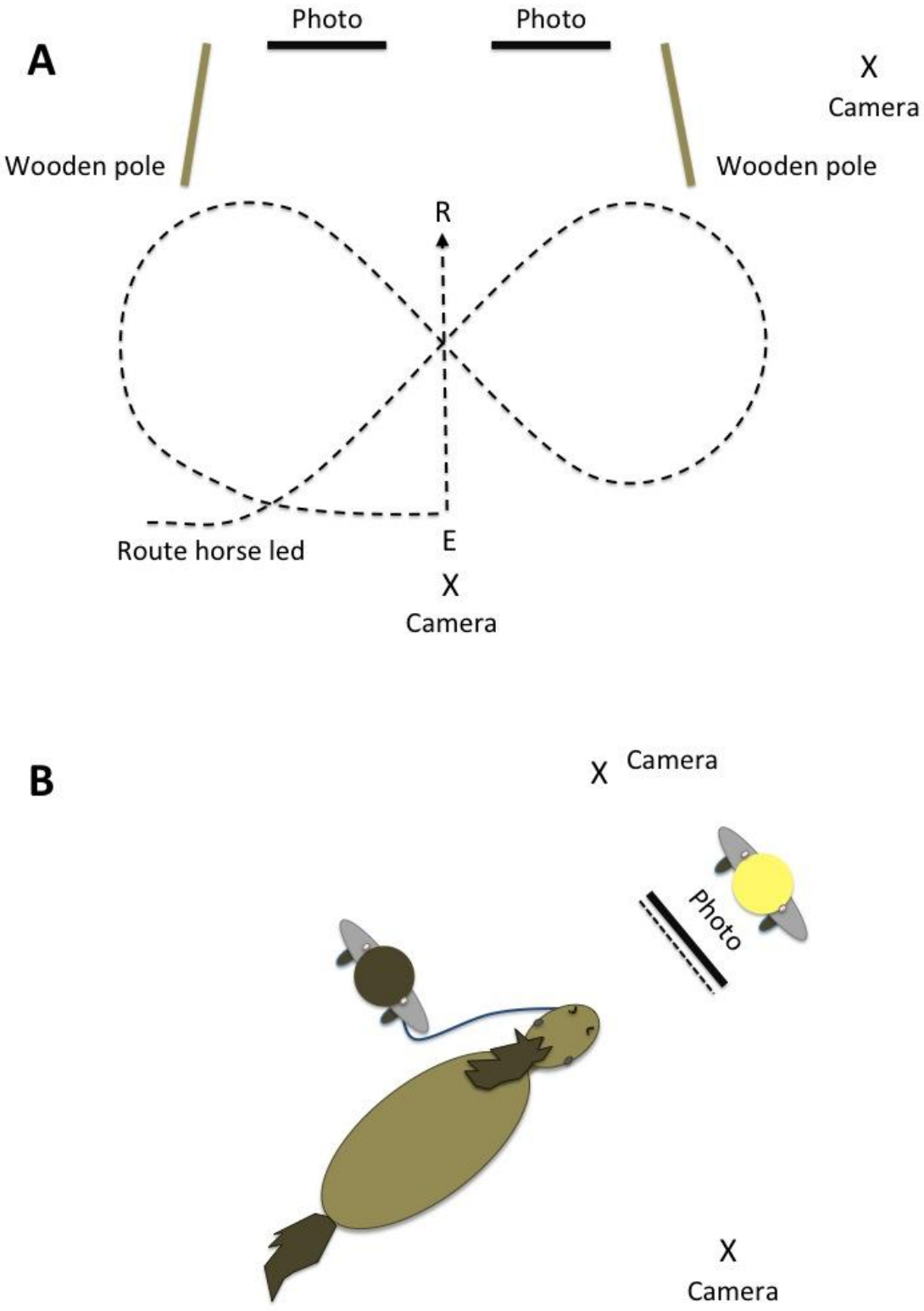


Figure 26. Diagrammatic representation of the experimental set up: A - experiment 1; B - experiment 2. The dotted line in panel B represents the markers on the floor used by the experimenter to ensure correct positioning of the stimulus being presented.

Behavioural analysis

Videos from two viewpoints were synchronized and analyzed for behavioural responses using Sportscode Gamebreaker Plus (www.sportstech.com). See Table 8 for definitions of the behaviours used for coding. In experiment 1, ten videos (21%) were analyzed by a second coder, revealing good to excellent levels of agreement: did the horse approach a photo (Cronbach's alpha = 1); which photo was approached first (Cronbach's alpha = 1); looking time at the photographs (two measures per trail; Spearman's rank correlation: n=20, r=.90, P<.001); time touching each photograph (two measures per trial; Spearman's rank correlation: n=20, r=.94, P<.001); proximity in each quarter of the test area (four measures per trial; Spearman's rank correlation: n=40: r=.85, P<.001). In experiment 2 the video coding was split equally between KG and JW: KG was aware of the photograph in the presentation, while JW was blind to the stimuli (achieved by using tools in the video software to occlude the stimuli on the screen). Twelve videos (22%) were double coded, revealing good to excellent levels of agreement measured by Spearman's rank correlation: looking direction (forward, left bias, right bias – three measures per trial), n=36, r=.99, P<.001; approach behaviours, n=12, r=.99, P<.001; avoidance behaviours, n=12, r=.93, P<.001; ear position (both ears forward, both ears backward, left ear forward/right ear backward. right ear forward/left ear backward – four measures per trial), n=48, r=.99, P<.001.

Statistical analysis

In experiment 1, which photograph horses approached first (if any) was assessed using two-tailed binomial tests. In 12 trials the horse entered the testing area but did not approach a photograph (positive/negative, n=5; neutral/negative, n=4; positive/neutral, n=3). Data for looking time and proximity were excluded for one horse in the positive-neutral comparison as his trial was cut short (after approaching a photograph) due to equipment failure. The looking behaviour and proximity measures were positively skewed and so were analyzed using Wilcoxon Signed-Rank tests. Although we measured time touching the photographs, too few horses touched the negative photographs (n=5 across all trials) to make statistical comparisons. In experiment 2 Wilcoxon Signed-Rank tests were also used to analyze whether the emotion displayed in the photographic presentations influenced the behavioural measures and two key measures of heart rate: the difference in the average heart rate between the baseline and

test period, and the time from the end of the test for the heart rate to return to baseline (Supplementary Material). We chose to examine the mode rather than the median or the mean, as the mode is influenced less by extreme values commonly seen in heart rate recordings. All p-values presented are two-tailed.

To investigate whether there were any effects of subject age and sex, model horse, side of stimulus presentation, and comparison of expressions on key behavioural variables in experiment one, these factors were entered as predictors alongside photograph expression in a Linear Mixed Model (using Log transformed data). We also employed GLMs to investigate potential effects of subject age and sex, model horse, and presentation order on changes in heart rate during the test and recovery time (although the data were non-normal visual examination of the residual plots showed satisfactory model fit). Model averaging was conducted to extract parameter β estimates and their 95% confidence intervals. The significance of predictor variables was assessed by whether the 95% confidence intervals overlapped zero. See Supplementary Material for full details of model selection.

Results

Experiment 1. Horses discriminate between facial expressions of other horses, and adjust their social behaviour accordingly

Horses discriminated the negative facial expression from the positive and neutral, and the information present in the facial expressions informed their decision about whether to approach an unfamiliar conspecific. In our paired choice tests, horses approached the positive expression significantly more often than the negative (n=11, K=11, P<.001), and the neutral expression significantly more than the negative (n=12, K=10, P=.04). They did not discriminate between the positive and neutral expressions however (n=13, K=6, P=1) (Figure 28). Horses preferred to look at the positive expression over the negative (*Mdn*=8.91 vs. *Mdn*=2.98, Z=-2.74, P=.006, r=.48) and the neutral expression compared to the negative (*Mdn*=8.41 vs. *Mdn*=2.40, Z=-2.53, P=.01, r=.45), although they showed no preference for looking at the positive versus the neutral expressions (*Mdn* = 5.18 vs. *Mdn*=4.53, Z=-0.11, P=.91). Horses also chose to spend more time in front of the positive expression than the negative (*Mdn*= 24.48 vs. *Mdn*=1.29, Z=-2.80,

P=.005, *r*=.49), and the neutral expression than the negative expression (*Mdn*=15.42 v.s *Mdn*= 0, *Z*=-2.33, *P*=.02, *r*=.41), however again showed no difference in the time they stood in front of the positive and neutral expressions (*Mdn*=11.87 vs. *Mdn*=11.84, *Z*=-0.66, *P*=.51). In addition, when horses were stood in front of the positive expression they were significantly more likely to spend time in close proximity to the photograph (<1.5m) than further away (>1.5m) (n=25, *Mdn*=23.33 vs. *Mdn*=3.47, *Z*=-3.26, *P*=.001, *r*=.46). When horses were stood in front of the neutral expression they also chose to spend time in close proximity to the image rather than further away (n=25, *Mdn*=13.44 vs. *Mdn*=2.43, *Z*=-2.61, *P*=.009, *r*=.37). However, this was not seen for the negative expression, where horses showed no preference for the half of the testing area containing the stimuli compared to further away (n=14, *Mdn*=6.50 vs. *Mdn*=2.58, *Z*=-0.91, *P*=.36).

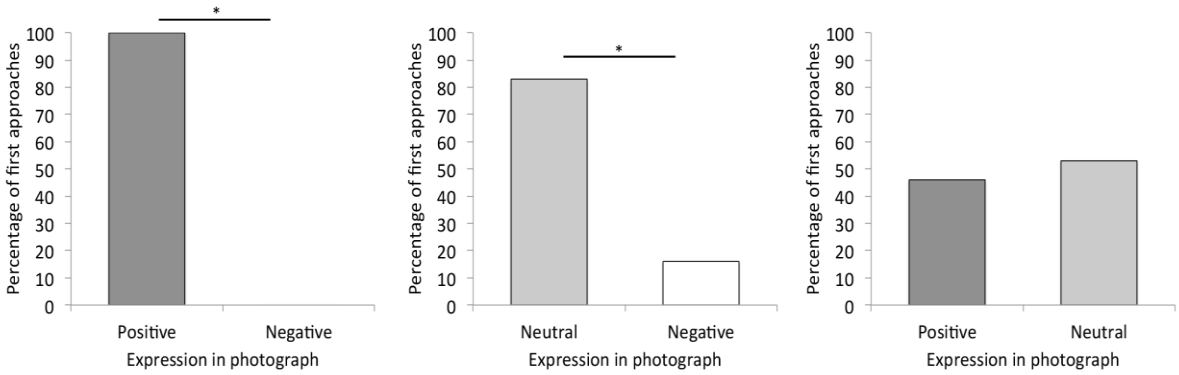


Figure 27. Choice of photographic stimuli approached first in experiment 1. Number of horses choosing to approach photographic stimuli depicting positive, neutral, and negative conspecific facial expressions in a series of paired choice tests. * represents a significant difference.

In the GLM analysis, the expression displayed in the photograph was a significant parameter and was featured in all of the top models (Table 10 and 11). This confirmed the results of the initial analysis (see above), demonstrating that horses showed no preference in proximity or looking time between the positive and neutral expressions, but chose to stand in front of and look at the negative expression significantly less (Table 10). Interestingly, younger horses spent more time overall looking at the photographs, but there was no interaction between age and expression, suggesting that

younger horses were generally more vigilant. Age did not significantly influence the horses' proximity to the expressions, and the 95% confidence intervals overlapped zero for all other potential predictors, indicating that they had no effect on looking behaviour or proximity (Table 10).

Experiment 2. Emotional facial expressions of conspecifics influence the behaviour and physiology of horses

The effect of facial expression on behaviour

In line with experiment 1, horses spent significantly more time actively avoiding the negative expression than they did the positive, with very few avoidance behaviours to the positive expression (*Mdn*=2.73 vs. *Mdn*= 0, *Z*=-2.11, *P*=.03, *r*=.03). More horses (*n*=12) engaged in approach behaviours to the positive expression compared to the negative expression (*n*=5), but numbers were small and statistical comparison was not possible.

Horses also showed different patterns in the way they chose to view the positive and negative expressions. Although there was no difference in binocular looking time to the positive and negative expressions (*Mdn*=23.02 vs. *Mdn*=22.81, *Z*=-0.36, *P*=.72), when viewing the negative expression horses displayed a bias for viewing it with their left eye compared to their right eye (*Mdn*=2.82, vs. *Mdn*=0.32, *Z*=-2.19, *P*=.03, *r*=.03). This was not seen as strongly when viewing the positive expression (*Mdn*=2.82 vs. *Mdn*=0, *Z*=-1.57, *P*=.12). Horses spent the same amount of time with their ears forward when viewing the positive expression and the negative expression (*Mdn*=21.6 vs. *Mdn*=20.52, *Z*=-0.71, *P*=.48), but more time with both ears angled backwards when viewing the positive expression compared to the negative expression (*Mdn*=4.35 vs. *Mdn*=2.81, *Z*=-2.4, *P*=.02, *r*=.04). Horses spent longer with their ears in an asymmetrical position (one forward one back) when viewing the negative expression than when viewing the positive expression (*Mdn*=5.4, vs. *Mdn*=3.8, *Z*=-2.4, *P*=.02, *r*=.04), however there was no preference for a specific asymmetrical ear combination in response to either of the expressions (p>.5 for all comparisons).

The GLM analysis confirmed our initial results (see above), showing that the facial expression displayed significantly influenced the time horses spent engaging in

avoidance behaviours but did not influence the time that horses spent (binocular) looking at the expressions (Table 12). Horses also spent significantly longer looking at model horse 1 than model horse 2 (horse 1 *M*=24.34, *SEM*=0.85; horse 2 *M*=19.34 *SEM*=1.42), although there was no significant difference in time spent avoiding the model horses (horse 1 *M*=1.60, *SEM*=0.40; horse 2 *M*=4.40, *SEM*=1.08). Interestingly, sex had an influence on behaviour; male horses looked at the stimuli for longer than females (males *M*=23.83, *SEM*=1.03; females *M*=19.68, *SEM*=1.33) but females were more reactive and spent more time avoiding the stimuli than males (males *M*=1.73, *SEM*=0.51; females *M*=4.42, *SEM*=1.08). Finally, there appeared to be a habituation effect, where horses spent significantly more time avoiding the stimuli in the first presentation than the second presentation (first *M*=4.14, *SEM*=0.97; second *M*=1.6, *SEM*=0.50); however it is notable that horses spent more time looking at the stimuli in the second presentation than the first (first *M*=20.39, *SEM*=1.26; second *M*=23.78, *SEM*=1.08).

The effect of facial expression on heart rate

In response to both emotional faces, the modal heart rate increased between the baseline period and the test period; however, modal heart rate increase was marginal in response to the positive expression in comparison with a significantly larger increase in response to the negative expression (*Mdn*=0.55 vs. *Mdn*=16.90, *Z*=-2.80, *P*=.005, *r*=.05). Horses also took less time to return to their baseline mode (measured from the end of the test) after viewing the positive expression than after viewing the negative expression (*Mdn*=4.2 vs. *Mdn*=28.00, *Z*=-2.30, *P*=.02, *r*=.04) (Figure 29). The secondary, GLM analysis, also confirmed this result (Table 12).

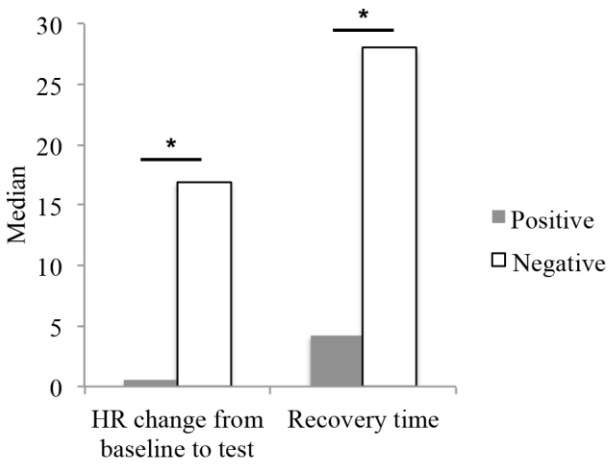


Figure 28. Average heart rate change in experiment 2 in response to presentation of the positive and negative photographs. The figure shows the change in average heart rate (bpm) between the baseline period and the test period, and the recovery time (in seconds) for heart rate to return the baseline mode after the end of the test period. * represents a significant difference.

Corresponding with the preference for looking at horse 1 seen in the behavioural data, horse heart rates were more elevated when exposed to horse 2 (horse 2 difference baseline-test: *M*=24.07, *SEM*=5.70; recovery time: *M*=31.28, *SEM*=5.74; horse 1 difference baseline-test: *M*=3.97, *SEM*=2.57; recovery time: *M*=10.57, *SEM*=2.64) (Table 12). There was also a significant effect of presentation order, with horses generally showing greater reactivity to the first presentation (baseline-test difference, *M*=18.12, *SEM*=4.98; recovery time, *M*=26.09, *SEM*=4.8) than the second presentation (baseline-test difference, *M*=7.69, *SEM*=3.8; recovery time, *M*=13.47, *SEM*=4.07). However there was no effect of sex, or any other predictor variables here (Table 12).

Discussion

Our results demonstrate that horses have the ability to spontaneously recognise and distinguish between emotional facial expressions of conspecifics in a functionally relevant way. In our experiments the photographs of positive and neutral facial expressions were clearly discriminated from the negative facial expressions, with the positive and neutral facial expressions eliciting more approach behaviours and the

negative expressions more avoidance behaviours. Such reactions would prove highly adaptive if horses were to encounter an unfamiliar conspecific displaying these facial expressions in their social environment, potentially increasing the opportunity for affiliative interactions and reducing the chance of conflict. Additionally, there were strong differences in the physiological reactions elicited by the emotional expressions, with average heart rate increasing more and taking longer to recover when viewing the negative facial expressions compared to the positive facial expressions. This provides insights into what the animals were experiencing and the underlying mechanisms that are likely to drive their social decision-making.

Despite a lack of research into the facial expressions in a wide range of species, it is frequently stated that primates produce and perceive facial expressions in a unique and more sophisticated way than other animals e.g. [10, 11-15]. However, here we provide experimental evidence demonstrating the use of facial expressions as sources of social information in an animal far removed from the primate lineage: the domestic horse. This contributes to recent evidence demonstrating previously underestimated capacity for the production of facial expressions in a range of domestic animals, including horses, that demonstrate a surprising number of similarities to some primates [21, 25, 26]. Given the sophisticated facial repertoire of the horse, it will now be interesting to establish to what extent their range of individual facial movements might be combined and used in ways beyond the basic discriminations we have investigated here. Our findings also support recent calls for scientists to extend their studies of facial expressions across a wider range of species to allow us to consider more fully the extent to which some facial expressions may be the result of shared ancestral characteristics or may offer some advantage in response to common selective pressures [3, 44].

Several socio-ecological variables have been suggested to be influential in the evolution of facial expressions, including arboreality, body size and social organisation [7, 45]. However arboreality does not significantly correlate with facial mobility in primates [3, 7], and while larger primate species have greater facial mobility, body size alone does not entirely explain expressive capability; when body size is controlled for, facial mobility increases in line with group size and complexity [4, 7, 11]. This corresponds with suggestions that facial expressions are rich sources of social information, and that

they might have a key role in the management of social relationships and group cohesion, which is particularly important in complex social networks [4-7].

However, it is currently difficult to come to firm conclusions about the function of facial expressions because of the narrow range of species that have previously been studied. Moreover, although it has been suggested that accurate recognition and discrimination of facial expressions plays a key role in the social communication and behaviour of non-human animals, no previous research has provided direct evidence to demonstrate the mechanisms through which this might be achieved. Here we provide experimental evidence of sensitivity to emotional facial expressions working as an adaptive mechanism in social networks by influencing internal states, behaviour and interactions with conspecifics in a functionally relevant way. The naturalistic design and spontaneous nature of our tests has demonstrated that the ability to discriminate between facial expressions has a socially facilitative purpose: i.e. informing a decision about whether to approach an unfamiliar conspecific. This indicates that facial expressions do make the behaviour of conspecifics more predictable, and potentially inform the receiver about the internal state of the signaller.

Crucially, our physiological data provides insights into how the horses experience the emotional facial expressions of a conspecific, and reveal their internal states when they are making these social decisions. The increased heart rate in response to the negative facial expressions, but minimal response to the positive facial expressions demonstrates that these expressions engendered corresponding emotional responses in the signal receiver. Whilst such changes may reflect learned associations, for example fear of a likely negative encounter with a conspecific displaying a negative facial expression, this could also demonstrate a level of emotional contagion, where the perception of an emotional state in another activates shared representations that cause a matching state in the observer [46]. These two concepts are certainly not mutually exclusive, and it has been proposed that emotional contagion might have a range of functions, including enhancing communication between conspecifics about the environment (i.e. whether safe or not), which here seems to include the social environment [47].

Intriguingly, although horses clearly distinguished the positive and neutral expressions from the negative expressions, they did not show clear behavioural discrimination

between positive and neutral. This has also been seen in chimpanzees, who did not discriminate a relaxed lip face from a neutral face, even though they could distinguish these faces from other expressions [6]. Whilst measurement of the horse facial expressions using EquiFACS demonstrated differences in the positive and neutral expressions, these were minimal and may not be distinguished in practice by horses [26]. Alternatively, in our experimental situation – determining whether to approach a potential social partner –there may be no functional value in differentiating between expressions showing positive anticipation and a neutral state. Neutral facial expressions may represent a positive state of relaxation or contentment, which has been shown to elicit the same beneficial effects on the cardiovascular system as mild joy [48, 49]. Understanding and identifying positive experiences in animals has been highlighted as a key research priority and further investigation of the facial correlates of positive emotional states in non-human animals is vital both for scientists interested in animal communication and those concerned with animal welfare [42].

Age and experience improves the recognition and appraisal of social cues in humans [50] and African elephants [51, 52]. In our study younger horses spent longer looking at the photographs compared to older horses. This is consistent with previous reports that younger horses spent more time looking at a novel object than older individuals and may reflect the increased time required by less experienced individuals to evaluate the situation [34, 53]. In addition to age, sex also influenced behaviour in our study. Males spent longer looking at the stimuli, regardless of emotion portrayed, whereas females were more reactive (spent more time avoiding the stimuli), perhaps due to the different roles the sexes play in free-ranging horse societies, where males take on a protective role for the group [38]. When viewing the emotional facial expressions, the horses in our study also showed a general gaze bias for viewing the stimuli with the left eye; however, this was only significant in response to the negative facial expressions. Lateralisation can provide important cues to the cognitive processes underpinning stimuli appraisal, and the left eye gaze bias suggests a right hemisphere dominance for processing the stimuli, which was stronger in response to the negative facial expressions. Across mammals the right hemisphere is generally specialised for novel and emotional stimuli, in particular the assessment of threatening situations [54-56]. The right hemisphere is also thought to be predominant in facial processing and identity recognition [54]. Thus the overall right hemispheric dominance could reflect the

activation of face processing centres, which was further enhanced when subjects were presented with the potentially threatening negative facial image.

To summarise, it has been suggested that the functional use of complex facial expressions as a means of social communication is restricted to the primate taxa, yet naturalistic studies of other species have been neglected [3]. Our results clearly show that horses gain important information from the facial expressions of others, which influences their social behaviour. These findings indicate that this ability may be far more widespread than previously thought, and systematic work incorporating naturalistic paradigms across a wide range of species with different social and ecological environments is now necessary to fully understand the prevalence, function, and evolution of facial expression. To progress, we also need to devise innovative paradigms that elucidate the cognitive and neurological mechanisms underpinning these interactions and to look directly at the fitness benefits animals may accrue from better communication. This is a major priority both for scientific and welfare purposes: a better understanding of what facial expressions mean, particularly those associated with emotion, could have very significant impacts on the management of captive, domestic, and even wild animals.

Supplementary Material

Supplementary methods

Stimuli Characterisation

The emotional expressions were characterised using the Equine Facial Action Coding System (EquiFACS)[1]. This is an anatomically based framework for describing facial expressions in horses. The stimuli were coded by two trained EquiFACS coders, one of whom was blind to the emotional context of the stimuli, purpose of the stimuli, and the experimental protocol. There was 96 % agreement on the codes. See Table 7 for a full description of the codes.

Table 7. Facial actions present in the photographic stimuli, as described using EquiFACS.

Emotional context	Model horse	EquiFACS codes
Positive	1	Ears forward (EAD101)
Positive	2	Right ear forward (EAD101, R), nostril dilation (AD38)
Neutral	1	Right ear forward (EAD101, R)
Neutral	2	0
Negative	1	Ear flattener (EAD103), Ear rotator (EAD104), Nostril lift (AUH13), Lip pucker (AU18)
Negative	2	Ear flattener (EAD103), Ear rotator (EAD104), Nostril lift (AUH13), Lip pucker (AU18)

Table 8. Definitions of behaviours used for coding.

Behaviour		Definition
Experiment 1		
Looking at photograph		Attentive with head orientated directly towards the photograph.
Approach		Horse walks up to the photograph, bringing their nose to within 30cm. If horses approached both photographs we measured which they approached first.
Touch the photograph		Horse touches the photograph with their nose.
Time spent in proximity to a photograph		Testing area divided into quarters. Horses' position measured from the time the first foreleg is placed into the quarter until the first foreleg leaves the quarter.
Experiment 2		
Looking at photograph		Attentive with head orientated directly towards the photograph
Gaze bias left/right		Oriented to the stimuli (judged by at least one ear still oriented towards the photograph) but with the head turned to one side. Gaze bias left refers to a preference for viewing with the left eye (i.e. head turn right) and gaze bias right a preference for the right eye (head turn left).
Avoidance		Horse moves away from the photograph with visible alarm, e.g. nostril flaring, wide eyes, tense muscles.
Approach		Horse reaches their nose or moves their body forward towards the photograph.
Touch the photograph		Horse touches the photograph with their nose.
Both ears forward		The openings of both ears are facing forward of the midline.
Both ears back		The openings of both ears are facing posteriorly of the midline.
Asymmetrical ears	Left ear forward/right ear back	The opening of the left ear is facing forward of the midline while the right ear opening is directed posteriorly.
	Right ear forward/left ear back	The opening of the right ear is facing forward of the midline while the left ear opening is directed posteriorly.

Heart rate data processing

Data were uploaded from the receiver watch using the Polar ProTrainer 5 Equine Edition software (Polar UK, Warwick, UK). The raw (inter-beat interval) data were then exported as .txt files and imported into Kubios HRV (v. 2.2; Biosignal Analysis and Medical Imaging Group, University of Eastern Finland, Kuopio, Finland). We applied custom artefact correction at 0.3, the number of corrections made was noted and then the corrected files were exported as .txt files for statistical analysis.

Identification of Heart Rate Recovery

Heart rate recovery was measured as the time taken from the end of the test for the heart rate to return to the baseline mode. This was achieved by examination of the raw heart rate data. For a horse to be considered as returning to the baseline mode, we ruled that five consecutive heart beats at baseline mode intervals must be observed, where only one of the five beats may be above the baseline mode by one integer. For example, if the baseline mode was 40 bpm, return could be classed when the heart beat record measured: 40, 40, 40, 41, 40.

Model selection and parameter estimates

Where linear mixed models were run, participant ID was included as a random factor in all models to account for repeated measurements from the same animals. Models were analysed using the lme4 package in R (v.3.1.1; R Foundation for Statistical Computing, Vienna, Austria). Candidate models were generated a priori based on the predictor variables. These included a null model (intercept only), a global model with all the potential predictors, and reduced models both including and excluding the main effect parameter included. See Table 7 for candidate models for experiments 1 and 2.

Akaike's Information Criterion adjusted for small sample size (AICc) was used for model selection [2]. The AICcmodavg package was used to extract AICc scores and model weights for candidate models of each response variable. Model averaging was conducted on the models accounting for at least 0.95 of the AICc weight to extract parameter β estimates and their 95% confidence intervals. The significance of predictor variables was assessed by whether the 95% confidence intervals overlapped zero. Interactions between significant variables were examined post-hoc, but no significant interactions were identified.

Table 9. Structure of the candidate models assessed for key dependent variables.

Experiment 1
Null
Expression+Comparison+Side of Stimuli+Sex+Age+Model horse seen+Expression*Side of Stimuli
Expression+Comparison+Side of stimuli+Sex+Age+Model horse seen
Expression +Side of stimuli
Expression +Sex
Expression +Age
Expression +Model horse seen
Expression+Comparison
Comparison+Side of stimuli+Sex+Age+Model
Comparison
Side of stimuli
Sex
Model horse seen
Age

Experiment 2
Null
Expression+Trial no+Model+Sex+Age
Expression+Trial no
Expression+ Model
Expression+ Sex
Expression +Age
Trial no+Model+Sex+Age
Trial no
Model
Sex
Age

Supplementary results

Table 10. The observed β estimates (± 95%CIs) for the model averaged parameters from the top models (experiment 1). Bold text denotes significant predictors (i.e. β estimates with 95% CIs that did not overlap zero).

	Parameter		β estimate	95% CIs
Looking Time	**Expression displayed (base category: neutral)**	Positive	0.01	-0.46, 0.49
		Negative	**-0.81**	**-1.29, -0.32**
	Age		**-0.04**	**-0.07, -0.01**
	Comparison of expressions (base category: neutral/negative)	Neutral/positive	-0.37	-0.91, 0.17
		Positive/negative	0.27	-0.39, 0.67
	Side of stimuli		0.22	-0.16, 0.6
	Model horse		0.19	-0.38, 0.38
	Sex		0.01	-0.52, 0.54
Proximity	**Expression displayed (base category: neutral)**	Positive	0.41	-0.71, 0.91
		Negative	-1.41	-2.32, -0.51
	Age		-0.04	-0.09, 0.02
	Comparison of expressions (base category: neutral/negative)	Neutral/positive	-0.88	-0.52, 0.72
		Positive/negative	0.07	-0.77, 0.92
	Side of stimuli		-0.41	-1.02, 0.2
	Model horse		0.1	-0.52, 0.72
	Sex		0.09	-0.67, 0.85

Table 11. Top models accounting for ≥ 0.95 of the AICc weight for looking time at the photographs and time stood in proximity to the photographs (experiment 1). K is the number of parameters in the model.

	K	ΔAICc	AICc Weight	Log Likelihood
Looking time				
Expression+Age	6	0	0.80	-124.50
Expression+Comparison	7	5.34	0.06	-126.00
Expression+Side of stimuli	6	5.51	0.05	-127.26
Expression+Model horse	6	6.71	0.03	-127.86
Expression+Sex	6	6.72	0.03	-127.86
Proximity				
Expression+Comparison	7	0	0.42	-168.67
Expression+Age	6	1.57	0.19	-170.62
Expression+Side of Stimuli	6	1.68	0.18	-170.68
Expression+Model horse	6	3.26	0.08	-171.47
Expression+Sex	6	3.32	0.08	-171.50
Expression+Comparison+Side of stimuli+Sex+Age+Model horse	11	6.22	0.02	-166.80

Table 12. The observed β estimates (± 95%CIs) for the model averaged parameters from the top models (experiment 2). Bold text denotes significant predictor variables (β estimates with 95% CIs that did not overlap zero).

	Parameter	β estimate	95% CIs
Time spent avoiding the photograph	**Expression**	**-1.58**	**-3.05, -0.11**
	Presentation	**-2.5**	**-3.99, -1.01**
	Sex	**2.43**	**0.22, 4.64**
	Model	-1.92	-4.17, 0.33
	Age	0.23	-0.03, 0.48
Time spent looking at the photograph	**Presentation**	**3.41**	**0.55, 6.26**
	Model	**4.26**	**1.15, 7.37**
	Sex	**-3.44**	**-6.44, -0.45**
	Expression	-0.79	-3.7, 2.12
	Age	-0.14	-0.49, 0.2
HR Difference from Baseline - Test	**Expression**	**-16.29**	**-25.8, -6.77**
	Presentation	**-11.03**	**-20.37, -1.68**
	Model	**-20.22**	**-30.37, -10.06**
	Sex	8.68	-1.13, 18.48
	Age	-0.95	-2.01, 0.1
HR Recovery Time	**Expression**	**-13.54**	**-22.57, -4.25**
	Presentation	**-13.08**	**-22.1, -4.06**
	Model	**-20.48**	**-31.53, -9.42**
	Sex	9.69	-1, 2, 0.39
	Age	-0.9	-2.05, 0.25

Table 13. Top models accounting for ≥ 0.95 of the AICc weight for key behavioural and heart rate measures in experiment 2. K is the number of parameters in the model.

	K	ΔAICc	AICc Weight	Log Likelihood
Avoiding time				
Emotion+Trial no+Model+Sex+Age	8	0	0.59	-179.09
Trial no+Model+Sex+Age	7	1.61	0.26	-181.19
Emotion+Trial no	5	3.83	0.09	-184.77
Trial no	4	5.68	0.03	-186.87
Looking time				
Trial no+Model+Sex+Age	7	0	0.56	-210.96
Emotion+Trial no+Model+Sex+Age	8	2.28	0.18	-210.81
Model	4	2.78	0.14	-215.99
Emotion+Model	5	4.91	0.05	-215.89
Sex	4	5.95	0.03	-217.58
Increase Baseline-Test				
Expression+Trial no+Model+Sex+Age	8	0	0.8	-231.07
Expression+Model	5	3.12	0.17	-236.60
HR Recovery time				
Expression+Trial no+Model+Sex+Age	8	0	0.82	-232.26
Expression+Model	5	4.83	0.07	-238.65
Trial no+Model+Sex+Age	7	4.96	0.07	-236.12

Supplemental Videos

Please contact j.wathan@sussex.ac.uk for the video files and their captions.

Acknowledgments

We would like to thank Chris Wathan, Amy Smith, Becky Spooner, and Andy Bamford for help with the data collection, and Andy and Stan Abrahams for statistical advice. Katie Slocombe and Amy Lyons contributed photographic stimuli, and Cátia Caerio performed the second EquiFACS coding. We are grateful to the staff and horses at all the equestrian centers that participated. JW was funded by a studentship from the Biotechnology and Biological Research Council (BB/F016808/1) and the University of Sussex; KM, LP, & KG, were funded by a research grant from the Leverhulme Trust (RPG-2013-069). The authors declare we have no competing interests.

References

Main text

1. Danchin E., Giraldeau L.A., Valone T.J., Wagner R.H. 2004 Public information: from nosy neighbors to cultural evolution. *Science* **305**(5683), 487-491. (doi:10.1126/science.1098254).
2. Hole G., Bourne V. 2010 *Face Processing: Psychological, Neuropsychological, and Applied Perspectives*, OUP Oxford.
3. Waller B.M., Micheletta J. 2013 Facial Expression in Nonhuman Animals. *Emotion Review* **5**(1), 54-59. (doi:10.1177/1754073912451503).
4. Dobson S.D. 2009 Allometry of facial mobility in anthropoid primates: implications for the evolution of facial expression. *Am J Phys Anthropol* **138**(1), 70-81. (doi:10.1002/ajpa.20902).
5. Waller B.M., Dunbar R.I. 2005 Differential behavioural effects of silent bared teeth display and relaxed open mouth display in chimpanzees. *Ethology* **111**, 129-142.
6. Parr L.A. 2003 The Discrimination of Faces and Their Emotional Content by Chimpanzees (Pan troglodytes). *Ann N Y Acad Sci* **1000**(1), 56-78. (doi:10.1196/annals.1280.005).
7. Dobson S.D. 2009 Socioecological correlates of facial mobility in nonhuman anthropoids. *Am J Phys Anthropol* **139**(3), 413-420. (doi:10.1002/ajpa.21007).
8. Aureli F., Schaffner Colleen M., Boesch C., Bearder Simon K., Call J., Chapman Colin A., Connor R., Fiore Anthony D., Dunbar Robin I.M., Henzi S.P., et al. 2008 Fission- Fusion Dynamics: New Research Frameworks. *Current Anthropology* **49**(4), 627-654. (doi:10.1086/586708).
9. Darwin C. 1999 *The Expression of the Emotions in Man and Animals*. London, UK, Fontana Press.
10. Huber E. 1931 *Evolution of facial musculature and expression*. Baltimore, John Hopkins Press.
11. Dobson S. 2012 Face to face with the social brain. *Philos Trans R Soc Lond B Biol Sci* **367**(1597), 1901-1908. (doi:10.1098/rstb.2011.0224).
12. Andrew R.J. 1963 Evolution of Facial Expression. *Science* **142**(3595), 1034-1041. (doi:10.1126/science.142.3595.1034).
13. Burrows A.M. 2008 The facial expression musculature in primates and its evolutionary significance. *BioEssays* **30**(3), 212-225. (doi:10.1002/bies.20719).
14. Tate A.J., Fischer H., Leigh A.E., Kendrick K.M. 2006 Behavioural and neurophysiological evidence for face identity and face emotion processing in animals. *Philos Trans R Soc Lond B Biol Sci* **361**(1476), 2155-2172. (doi:10.1098/rstb.2006.1937).
15. Boissy A., Aubert A., Desire L., Greiveldinger L., Delval E., Veissier I. 2011 Cognitive sciences to relate ear postures to emotions in sheep. *Animal Welfare* **20**(1), 47.
16. Burrows A.M., Diogo R., Waller B.M., Bonar C.J., Liebal K. 2011 Evolution of the Muscles of Facial Expression in a Monogamous Ape: Evaluating the Relative Influences of Ecological and Phylogenetic Factors in Hylobatids. *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology* **294**(4), 645-663. (doi:10.1002/ar.21355).
17. Burrows A.M., Smith T.D. 2003 Muscles of facial expression in Otolemur, with a comparison to lemuroidea. *Anat Rec A Discov Mol Cell Evol Biol* **274**(1), 827-836. (doi:10.1002/ar.a.10093).

18. Burrows A.M., Waller B.M., Parr L.A. 2009 Facial musculature in the rhesus macaque (Macaca mulatta): evolutionary and functional contexts with comparisons to chimpanzees and humans. *Journal of Anatomy* **215**(3), 320-334. (doi:10.1111/j.1469-7580.2009.01113.x).
19. Burrows A.M., Waller B.M., Parr L.A., Bonar C.J. 2006 Muscles of facial expression in the chimpanzee (Pan troglodytes): descriptive, comparative and phylogenetic contexts. *Journal of Anatomy* **208**(2), 153-167. (doi:10.1111/j.1469-7580.2006.00523.x).
20. Diogo R., Wood B.A., Aziz M.A., Burrows A. 2009 On the origin, homologies and evolution of primate facial muscles, with a particular focus on hominoids and a suggested unifying nomenclature for the facial muscles of the Mammalia. *Journal of Anatomy* **215**(3), 300-319. (doi:10.1111/j.1469-7580.2009.01111.x).
21. Waller B.M., Peirce K., Caeiro C.t.C., Scheider L., Burrows A.M., McCune S., Kaminski J. 2013 Paedomorphic Facial Expressions Give Dogs a Selective Advantage. *PLoS one* **8**(12), e82686. (doi:10.1371/journal.pone.0082686).
22. Ekman P., Friesen W.V., Hager J.C. 2002 *Facial Action Coding System*. Salt Lake City, Research Nexus.
23. Vick S.J., Waller B.M., Parr L.A., Smith Pasqualini M.C., Bard K.A. 2007 A Cross-species Comparison of Facial Morphology and Movement in Humans and Chimpanzees Using the Facial Action Coding System (FACS). *J Nonverbal Behav* **31**(1), 1-20. (doi:10.1007/s10919-006-0017-z).
24. Parr L.A., Waller B.M., Burrows A.M., Gothard K.M., Vick S.J. 2010 MaqFACS: A muscle-based facial movement coding system for the rhesus macaque. *Am J Phys Anthropol* **143**(4), 625-630. (doi:10.1002/ajpa.21401).
25. Caeiro C.C., Burrows, A.M., Waller, B.M. 2013 CatFACS. (Univeristy of Portsmouth.
26. Wathan J., Burrows A., Waller B.M., McComb K. 2015 EquiFACS: the Equine Facial Action Coding System. *PLOS One* **in press**.
27. Schilder M.B.H., van Hooff J.A.R.A.M., van Geer-Plesman C.J., Wensing J.B. 1984 A Quantitative Analysis of Facial Expression in the Plains Zebra. *Zeitschrift für Tierpsychologie* **66**(1), 11-32. (doi:10.1111/j.1439-0310.1984.tb01352.x).
28. Racca A., Guo K., Meints K., Mills D.S. 2012 Reading faces: differential lateral gaze bias in processing canine and human facial expressions in dogs and 4-year-old children. *PLoS one* **7**(4), e36076. (doi:10.1371/journal.pone.0036076).
29. Micheletta J., Whitehouse J., Parr L.A., Waller B.M. 2015 Facial expression recognition in crested macaques (Macaca nigra). *Anim Cogn*, 1-6. (doi:10.1007/s10071-015-0867-z).
30. Müller Corsin A., Schmitt K., Barber Anjuli L.A., Huber L. 2015 Dogs Can Discriminate Emotional Expressions of Human Faces. *Current Biology*. (doi:10.1016/j.cub.2014.12.055).
31. Nagasawa M., Murai K., Mogi K., Kikusui T. 2011 Dogs can discriminate human smiling faces from blank expressions. *Anim Cogn* **14**(4), 525-533. (doi:10.1007/s10071-011-0386-5).
32. Nakashima S.F., Ukezono M., Nishida H., Sudo R., Takano Y. 2015 Receiving of emotional signal of pain from conspecifics in laboratory rats. *Royal Society Open Science* **2**(4). (doi:10.1098/rsos.140381).
33. Sotocinal S.G., Sorge R.E., Zaloum A., Tuttle A.H., Martin L.J., Wieskopf J.S., Mapplebeck J.C., Wei P., Zhan S., Zhang S., et al. 2011 The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain* **7**, 55. (doi:10.1186/1744-8069-7-55).

34. Proops L., McComb K., Reby D. 2009 Cross-modal individual recognition in domestic horses (Equus caballus). *Proc Natl Acad Sci U S A* **106**(3), 947-951. (doi:10.1073/pnas.0809127105).
35. Heitor F., do Mar Oom M., Vicente L. 2006 Social relationships in a herd of Sorraia horses Part II. Factors affecting affiliative relationships and sexual behaviours. *Behav Processes* **73**(3), 231-239. (doi:10.1016/j.beproc.2006.05.005).
36. Cozzi A., Sighieri C., Gazzano A., Nicol C.J., Baragli P. 2010 Post-conflict friendly reunion in a permanent group of horses (Equus caballus). *Behav Processes* **85**(2), 185-190. (doi:10.1016/j.beproc.2010.07.007).
37. Emery N.J. 2005 The evolution of social cognition. In *The Cognitive Neuroscience of Social Behaviour* (eds. Easton A., Emery N.J.). Hove, UK, Psychology Press.
38. Waring G.H. 2003 *Horse Behavior*, Noyes Publications/William Andrew Pub.
39. Klingel H. 1977 Communication in Perissodactyla. In *How Animals Communicate* (ed. Sebeok T.A.). Bloomington & London, Indiana University Press.
40. Wathan J., McComb K. 2014 The eyes and ears are visual indicators of attention in domestic horses. *Curr Biol* **24**(15), R677-679. (doi:10.1016/j.cub.2014.06.023).
41. Mendl M., Burman O.H., Paul E.S. 2010 An integrative and functional framework for the study of animal emotion and mood. *Proc Biol Sci* **277**(1696), 2895-2904. (doi:10.1098/rspb.2010.0303).
42. Boissy A., Manteuffel G., Jensen M.B., Moe R.O., Spruijt B., Keeling L.J., Winckler C., Forkman B., Dimitrov I., Langbein J., et al. 2007 Assessment of positive emotions in animals to improve their welfare. *Physiology & Behavior* **92**(3), 375-397. (doi:10.1016/j.physbeh.2007.02.003).
43. von Borell E., Langbein J., Despres G., Hansen S., Leterrier C., Marchant-Forde J., Marchant-Forde R., Minero M., Mohr E., Prunier A., et al. 2007 Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals -- a review. *Physiology & Behavior* **92**(3), 293-316. (doi:10.1016/j.physbeh.2007.01.007).
44. de Waal F.B. 2011 What is an animal emotion? *Ann N Y Acad Sci* **1224**, 191-206. (doi:10.1111/j.1749-6632.2010.05912.x).
45. Kingdon J. 2007 Primate Visual Signals in Noisy Environments. *Folia Primatologica* **78**(5-6), 389-404.
46. de Waal F.B. 2008 Putting the altruism back into altruism: the evolution of empathy. *Annual Review of Psychology* **59**, 279-300. (doi:10.1146/annurev.psych.59.103006.093625).
47. Vallacher R.R., Nowak A., Zochowski M. 2005 Dynamics of social coordination: The synchronization of internal states in close relationships. *Interaction Studies* **6**(1), 35-52.
48. Fredrickson B.L. 2004 The broaden-and-build theory of positive emotions. *Philos Trans R Soc Lond B Biol Sci* **359**(1449), 1367-1378. (doi:10.1098/rstb.2004.1512).
49. Fredrickson B.L., Levenson R.W. 1998 Positive Emotions Speed Recovery from the Cardiovascular Sequelae of Negative Emotions. *Cogn Emot* **12**(2), 191-220. (doi:10.1080/026999398379718).
50. Grossmann I., Na J., Varnum M.E., Park D.C., Kitayama S., Nisbett R.E. 2010 Reasoning about social conflicts improves into old age. *Proc Natl Acad Sci U S A* **107**(16), 7246-7250. (doi:10.1073/pnas.1001715107).

51. McComb K., Shannon G., Durant S.M., Sayialel K., Slotow R., Poole J., Moss C. 2011 Leadership in elephants: the adaptive value of age. *Proc Biol Sci* **278**(1722), 3270-3276. (doi:10.1098/rspb.2011.0168).
52. McComb K., Moss C., Durant S.M., Baker L., Sayialel S. 2001 Matriarchs as repositories of social knowledge in African elephants. *Science* **292**(5516), 491-494.
53. Baragli P., Vitale V., Banti L., Sighieri C. 2014 Effect of aging on behavioural and physiological responses to a stressful stimulus in horses (Equus caballus). *Behaviour* **151**(11), 1513-1533.
54. MacNeilage P.F., Rogers L.J., Vallortigara G. 2009 Origins of the left & right brain. *Scientific American* **301**(1), 60-67.
55. De Boyer Des Roches A., Richard-Yris M.A., Henry S., Ezzaouia M., Hausberger M. 2008 Laterality and emotions: visual laterality in the domestic horse (Equus caballus) differs with objects' emotional value. *Physiology & Behavior* **94**(3), 487-490. (doi:10.1016/j.physbeh.2008.03.002).
56. Farmer K., Krueger K., Byrne R.W. 2010 Visual laterality in the domestic horse (Equus caballus) interacting with humans. *Anim Cogn* **13**(2), 229-238. (doi:10.1007/s10071-009-0260-x).

Supplemental material

1. Wathan J., Burrows A., Waller B.M., McComb K. 2015 EquiFACS: the Equine Facial Action Coding System. *PLOS One* **in press**.
2. Burnham K.P., Anderson D.R. 2002 *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, Springer.

Discussion

The work presented in this thesis has explored the transfer of information in social communication, through the production and perception of facial expressions in domestic horses. This has revealed that horses have the capacity to produce an extensive range of facial movements, and that the facial displays of horses are valuable sources of information to conspecifics. Moreover, by exploring the production of facial expressions in response to conspecific affective calls, and the cardiovascular responses to the perception of conspecific affective facial expressions, this research has shed light on some of the cognitive and physiological mechanisms underpinning the transfer of emotional information.

Much of the previous research investigating the production and perception of facial expressions had been conducted in primates. Whilst these results have been considered in an evolutionary framework, it has been difficult to fully evaluate the relevant ideas with evidence from only one taxon. This thesis, therefore, constitutes a valuable contribution to the literature by providing a range of comparable studies in a species that is phylogenetically distant from the primates. Moreover, as horses have simple foraging requirements but a rich and dynamic social environment, the study species is well placed to allow us to evaluate the relative influences of social and ecological factors on their communicative and cognitive abilities. Subsequently, the empirical work presented in this thesis allows us to consider some more general concepts around the likely influences of sociality on the evolution of sophisticated communication and cognition.

Overall, the results of this thesis contribute to knowledge of three broad areas of social cognition: the evolution of advanced and sophisticated communication systems, specifically facial expressions; how social sensitivity assists animals to navigate their environment; and, the cognitive and physiological mechanisms that underpin information transfer in social communication. These broad areas will be discussed in turn below, before drawing some final conclusions.

Evolution of a Complex Communication System: Facial Expressions

There is growing evidence that the challenges animals face in navigating their social environment may have been influential in the evolution of communication and cognition (Byrne and Whiten, 1988, Byrne and Bates, 2007, Dunbar, 2003, Humphrey, 1976, Jolly, 1966). Living in established social groups may have a range of advantages and hence is seen in a number of species, but it is a demanding option as competition for resources can have individual costs and disrupt group stability (Byrne and Bates, 2007, Dunn et al., 1995, Pusey and Packer, 2003). Consequently, for group living animals there is a need to regulate social interactions, maintain valuable relationships, and keep track of a complex network of associates in a competitive and dynamic environment. This suggests that efficient and sophisticated communication systems for the transfer of information might be advantageous, and examining the communicative repertoires in a range of species with different social, ecological, and phylogenetic histories can help us evaluate the extent to which different selective pressures may have been influential (Aureli et al., 2008, Byrne, 1995, Byrne and Bates, 2007, Cheney and Seyfarth, 2008, McComb and Semple, 2005, Premack and Woodruff, 1978).

Vocal communication has been well studied in a number of species, supported by systematic frameworks that allow the quantification of signals in relation to their anatomical basis, the ability to present realistic animal models in playback experiments, and the development of digital techniques for signal analysis and manipulation (Cheney and Seyfarth, 2008, Reby and McComb, 2003, Taylor and Reby, 2010). However, the study of visual communication does not yet have this extensive infrastructure, particularly for non-human animals. In this thesis I have advanced our current knowledge of visual communication, and our future ability to study this, through developing a new anatomically based method for the objective recording and analysis of facial expressions in the horse. This has established that horses have the capacity to use their faces to produce complex expressions, and has given us a quantifiable framework with which direct cross-species comparisons can be made. Furthermore, using recent advances in digital photography I have developed naturalistic experimental paradigms where visual signals could be systematically presented to examine what information receivers gained from these signals and how this influenced their behaviour.

Faces are important sources of social information (Bruce and Young, 2013, Hole and

Bourne, 2010). Mammals have a network of specialised facial muscles with extensive connections to the skin and superficial fascia, which enables these muscles to create observable changes in the shape of the face (Liem and Walker, 2001). These facial movements are produced in a range of different social contexts and can vary predictably with the producer's internal state (e.g. Chevalier-Skolnikoff, 1973, Darwin, 1999, Ekman and Friesen, 1971, Ekman et al., 1969). Thus, facial expressions have the potential to provide clues to the likely intentions and motivation of another individual, and could help to mediate social interactions (Andrew, 1963, Dobson, 2009a, Dobson, 2009b, Flack and de Waal, 2007, Parr, 2003, Parr et al., 2005, Waller and Dunbar, 2005). Consequently, the abilities to produce and accurately perceive varied facial expressions are likely to be highly valuable adaptive skills, particularly if social challenges are a factor driving the evolution of complex cognitive and communicative systems (McComb and Semple, 2005, Schmidt and Cohn, 2001).

There has been a long standing view that the ability to produce and perceive complex and meaningful facial expressions is confined to the primate order, with other animals having a very limited capacity (Andrew, 1963, Burrows, 2008, Boissy et al., 2011, Dobson, 2012, Huber, 1931, Tate et al., 2006). However, the work presented in this thesis provides solid evidence to refute that hypothesis, demonstrating that horses are also able to produce and perceive complex and meaningful facial expressions. From the findings presented in Article I (*EquiFACS: the Equine Facial Action Coding System*), it is clear that horses have an extensive and robust network of facial muscles with which they can produce a wide range of facial movements. As these are now documented in a way that allows us to make direct, cross-species comparisons, we can see that although this capacity is less well developed than that of humans, it is at least equivalent to that of other primates, including chimpanzees, and demonstrates a surprising degree of resemblance in some cases (Caeiro et al., 2012, Ekman et al., 2002, Parr et al., 2010, Vick et al., 2007, Waller et al., 2012). Moreover, the experimental work presented in Articles III and IV (*The Eyes and Ears are Visual Indicators of Attention in Domestic Horses; Functional use of Facial Expressions in a Non-Primate: Horses Discriminate Between Differing Emotions*) shows that horses are sensitive to detailed cues in the facial expressions of other horses and this information influences their behaviour in beneficial ways. Consequently, we need to reconsider our traditional conception of facial expressions and with this, the selective pressures that may have driven the

evolution of such a communicative system.

It is possible that elaborate facial architecture is an evolutionarily ancient trait, which is highly conserved across taxa (Waller and Micheletta, 2013). However it would be surprising if the ancestral form were so specialised, and more likely that it would have presented in a simpler arrangement consisting of thinner, flat, and undifferentiated sheets of muscles (Huber, 1931). Interestingly, some primates do show instances of facial muscles of this form. Around the ear, *Otolemur* (who are largely nocturnal and generally live fairly solitary lives) have a retrahens muscle that presents as a flat sheet with three bands of specialisation (Bearder et al., 2006, Burrows and Smith, 2003). In contrast, humans, chimpanzees, *Hylobatids*, rhesus macaques, and lemuroids have clearly differentiated superior and anterior auricularis muscles, as does the horse (Burrows, 2008, Burrows et al., 2011, Burrows et al., 2006, Burrows and Smith, 2003, Burrows et al., 2009, Huber, 1931). Whilst it could be that the ear muscles of *Otolemur* have specialised in response to some, as yet unknown, evolutionary pressures, it seems more parsimonious that they are typical of the ancestral arrangement, from which the similar ear muscles of some other primates and the horse have converged under common selective pressures. Humans, chimpanzees, *Hylobatids*, rhesus macaques, lemuroids and horses are all diurnal, but otherwise these animals occupy vastly differing ecological niches so it seems unlikely that physical demands have driven this evolution. However, these species all have to maintain valuable social relationships, and most must do so in a complex and dynamic social environment. The *Hylobatids* are an exception, living in small family groups; however their muscles are correspondingly less well developed and more gracile (Burrows et al., 2011). This evidence may be taken to support the idea that social factors are influential in the evolution of complex communicative systems. However, given the lack of comparable data about both capacity and use of facial expression in a wide range of species it is premature to draw firm conclusions about the evolutionary history of these traits.

Ear movements are one area where horses clearly diverge from many primates. Horses have large mobile ears, which Article III (*The Eyes and Ears are Visual Indicators of Attention in Domestic Horses*) demonstrates are an important visual communicative cue. In contrast, the apes cannot move their ears, rhesus macaques and lemuroids are capable of some ear movement, although their movements are less refined and their pinna less

exaggerated than horses, and *Otolemur* have large ears that they can move independently, demonstrating the most resemblance to horses (Ekman et al., 2002, Parr et al., 2010, Vick et al., 2007). It is thought that ear perking was a key communicative cue for our ancestors, and that other facial movement was limited because the ear musculature used to have connections to the rest of the face - as is still seen in *Otolemur* (Ekman and Oster, 1979, Rinn, 1984). Consequently, it has been proposed that functional differentiation of the ear and orbital muscles has evolved in some primates (e.g. humans and chimpanzees) to allow an expansion in the ability of the face to produce movement (Burrows and Smith, 2003, Ekman and Oster, 1979, Rinn, 1984, Waller and Dunbar, 2005, Waller et al., 2008, Waller, 2006). Alongside this it seems that humans and to a lesser degree some other primates, such as chimpanzees, have become specialised for the use of the eyes as a communicative cue, with brow movements taking the role of ear perking, particularly in accentuating or indicating attention (Ekman and Oster, 1979, Emery, 2000, Kobayashi and Kohshima, 2001, Kobayashi and Kohshima, 1997, Rinn, 1984). As the muscles of the ears and eyes have no connections to the rest of the facial musculature in horses, potentially this functional differentiation has also occurred to expand the capacity for facial movements. However, rather than specialising for cues in the eye region alone, for horses cues from both the eyes and the ears are important for visual communication and must be adaptive in their environment.

Similarly, this functional differentiation of the facial muscles is believed to have allowed the specialisation of the lips of humans to aid social signalling in a complex social environment (Burrows et al., 2006, Schmidt and Cohn, 2001). This was considered to be a highly unique feature for humans, however as with other apparently unique human traits it is now being demonstrated in other species. The results in Article I (*EquiFACS: the Equine Facial Action Coding System*) clearly demonstrate that horses have a very specialised lower face with an exceptional capacity for lip movements. In part, this may well be because horses do not have opposable digits for manipulating their environment, and the lips are their main tool for environmental exploration. However, horses also use their lower face to produce meaningful facial expressions. The development of EquiFACS has allowed us to identify that the lip retraction seen in the ‘snapping’ display of horses is underpinned by the zygomatic major, which also underpins the lip retraction seen in the primate bared teeth submissive gesture

(McDonnell, 2003, Parr et al., 2007, Waring, 2003). These expressions are given in similar contexts across the species, although it remains to be seen if they have similar social outcomes. Thus, my results demonstrate how studying the physiological underpinnings of expressive signals can offer us objective and quantifiable data on the capacities of animals, through which we can make direct, cross-species comparisons (Bolwig, 1964, Ekman et al., 2002, Ekman et al., 1990, Parr et al., 2007, Seyfarth and Cheney, 2003, Taylor and Reby, 2010, Vick et al., 2007).

Why are these expressions similar?

From the work presented here, it is clear that at least two animal taxa with distant phylogenetic relationships, varying ecological environments, and some substantial differences in their facial morphology have a sophisticated capacity to communicate through the use of complex facial expressions. However, the facial repertoires in these species have not evolved in a seemingly arbitrary manner, but rather in some cases very different animals display comparable expressions underpinned by similar musculature. This poses the interesting question: why are these particular expressions similar in such diverse species? Potentially the changes in facial morphology created in these displays act on inherent sensory biases common to all species, which make them adaptive and promote their selection. These common sensory biases may be adaptive in part because they enable cross-species as well as intra-species communication. This could possibly enable quicker recognition of a predator's internal states, and would also likely enhance communication in a domestic environment.

A general tendency seen across species is an increase in the apparent size of the orbital cavity in negative emotional situations. Many animals will widen the eyes in negative situations, possibly to optimise the use of visual perceptual resources. In some animals this also leads to an increase in the visible white sclera (e.g. humans, cows, and horses), which contrasts with the darker pupil and iris of the eye. This visual cue is easily perceptible supporting rapid information transfer in threatening situations, and in humans this cue is perceived rapidly even when the presentation is masked from conscious awareness (Whalen et al., 2004). This potential adaptive value has been proposed as a driver for the exaggeration of this feature that we see in humans (Emery, 2000, Kobayashi and Kohshima, 2001, Kobayashi and Kohshima, 1997); however, the

salience of this visual signal in other animals remains unknown.

In negative situations, humans and horses also raise the skin above the eye (AU101, inner brow raiser in EquiFACS; AU1, inner brow raiser in human FACS; S. Hintze, in prep., Waller et al., 2008). A recent study has demonstrated that in domestic dogs, the analogous expression was the key factor predicting speed of rehoming from rescue shelters, and the more dogs displayed this expression the faster they were rehomed (Waller et al., 2013). It was suggested that this movement alters the proportions of the face in a way that increases the apparent size of the orbital area, and may tap into human sensory preferences for neonatal proportions. It could be that the enlargement of the eye area that is typical in cross-species fearful/sadness situations (those likely requiring need and eliciting care) might exploit sensory biases that solicit care and help. A social recruitment function, such as this, may make signals that give away potentially costly information about the signaller's internal state pay in evolutionary terms. It has been argued that suppression of honest signals is common to avoid potential costs in both giving and receiving signals. This might be particularly expected in the case of animals that have previous experience of each other (Dawkins & Guilford, 1991). Future experiments investigating the effects of previous experience and relationship between signal giver and audience would be interesting to investigate these ideas further.

Other conspicuous similarities are the highly ritualised bared teeth submissive gesture and play faces seen in a range of species: both of which are indicators of benign intent, appeasement, and a wish for affiliation (Waller and Dunbar, 2005). The play face is characterised by an open mouth with the lips partially retracted and often covering the teeth (Fox, 1970, Parr et al., 2007, Pellis and Pellis, 1997, Poole, 1978, Reed and Brown, 2000). In the bared teeth submissive face the mouth is also open with the lips retracted (the lips will often be fully retracted), the teeth displayed, and sometimes the upper lip raised and the eyes narrowed (Parr et al., 2007, Preuschoft and van Hooff, 1997, Waller and Dunbar, 2005). The eye narrowing seems to be one of the features that distinguishes this display from aggressive faces, which often contain the same lip movements (Parr et al., 2007). Interestingly, eye narrowing is also a critical feature in distinguishing a genuine smile from a 'fake' smile in humans (Parr et al., 2007, Ekman et al., 1990). Thus it seems that subtle differences in similar expressions may aid the

discrimination of facial expressions given in different contexts, and produce different social outcomes.

Facial expressions are complex and nuanced sources of information, as the wide repertoire of facial movements can be combined in numerous different ways providing a graded continuum of signals. However, there is evidence that facial expressions may be perceived categorically by animals (Bruce and Young, 2013, Hole and Bourne, 2010, Parr, 2011, Parr, 2003). Categorical perception is widespread in human cognition and is considered to be a mechanism through which we can organize and process the vast quantities of information that we perceive; colour recognition is an example of a graded continuum that we perceived categorically (Harnad, 2003), Perhaps there are sensory biases in perceivers to detect subtle differences within a graded continuum of facial movements and link these with discrete events, such as an emotion.

Section summary

For humans, facial expressions are an important form of communication, but the extent to which this was true for other animals, and particularly non-primate species, was largely unknown. Yet, there is growing interest in this field about the selective pressures that have shaped the evolution of visual communication through facial expressions, which cannot be answered without the ability to make direct comparisons across a broad range of taxa. The work presented in this thesis has documented the ability of horses to produce and perceive facial expressions, and thus provided new evidence that contributes to our ability to consider the evolution of this complex communication system. This has demonstrated that an animal far removed from the primate order also has a sophisticated visual communication system in the form of facial expressions, which helps individuals to navigate their environment in functionally relevant ways. These complex communication systems show some areas of similarity, potentially in response to common selective pressures including the need to maintain valuable social relationships in a dynamic and fluid environment. Moreover, these signals potentially tap into sensory biases of the receiver that enhance the efficiency of these cues for information transfer and social recruitment.

The above evidence corresponds with the idea that facial expressions could help to mediate social interactions and could act, as language does, as a tool that can be used to access the mind of another (Goody, 1997). Through communication animals can express how they see the world and others in it, and studying such exchanges offers an insight into their social cognition. In the next section I go on to discuss what my findings demonstrate about the sensitivity of horses to the internal states of their conspecifics, and how this might help them navigate their social environment.

Social Sensitivity

Other animals can present a multitude of problems: they are complex organisms with continuously and rapidly changing needs and motivation (Humphrey, 1976). Yet, social partners can also have their advantages – they might give you advanced warning about predators in your environment, for example – and in a number of species having strong social bonds correlates significantly with survival (Cameron et al., 2009, Nunez et al., 2014, Silk et al., 2009, Silk et al., 2010). Therefore in social species, navigating your environment involves dealing with a number of individuals with whom you must retain relationships despite competition for resources. This presents a serious challenge, and consequently having a refined sensitivity to other animals and their internal states might be advantageous – potentially making the motivations and actions of others more predictable and allowing an individual to adjust their own behaviour accordingly. Indeed, for species in which life involves a need to sustain valuable social relationships it seems that natural selection has favoured animals that can represent, organise, and classify other individuals, their relationships, and their motivations (Aureli et al., 2008, Byrne and Whiten, 1988, Byrne and Bates, 2007, Cheney and Seyfarth, 2008, Humphrey, 1976, Jolly, 1966).

However, to be able to fully evaluate these ideas within an evolutionary framework, we need to understand more about how sensitivity to others might help animals to navigate their environment, including their social environment, and exactly what advantages these skills confer (Cheney and Seyfarth, 2008, Seyfarth et al., 2010). Naturalistic experimental paradigms that allow the systematic presentation of animal models and observation of the recipient's behaviour are the most effective way of doing this for valid results. Moreover, studying how animals interact with conspecifics can offer

insights into how they perceive their world and the cognitive mechanisms underpinning these interactions. Recent advances in digital photography have made the presentation of models in the visual modality more achievable, and computer based tasks presented to captive animals are beginning to give us some insights into how animals perceive visual signals from others (Parr, 2011). In my thesis I have taken this paradigm out of the lab and used photographic stimuli to present images of animal models in naturalistic settings. In doing this I was able to ask questions about not only how horses perceived their conspecifics, but also how the information gained from these signals directly influenced horse behaviour.

Horses are unusual as they are highly sociable and gregarious creatures who will rapidly approach conspecifics even if they are unfamiliar, but are otherwise extremely neophobic (Leiner and Fendt, 2011, McDonnell, 2003, Waring, 2003). This provided the ideal opportunity to validate the use of photographic stimuli as a method of presenting conspecific models, through comparing the reactions of horses to these stimuli and appropriately matched novel objects (phase scrambled versions of the stimuli). If horses perceived the stimuli as a depiction of another horse, then clear differences would be predicted in their reactions to the photographic models and novel objects. Indeed, findings presented in Article III (*The Eyes and Ears are Visual Indicators of Attention in Domestic Horses*) demonstrate that horses were significantly more likely to approach the horse stimuli and avoid the phase-scrambled controls, suggesting that horses do recognise the stimuli as the depiction of a horse and validating the use of photographs as a naturalistic methodology. I was therefore justified in using photographic models as a tool to explore whether horses were sensitive to the internal states of their conspecifics in two key areas: attention and emotion. These are discussed in turn below

Are horses sensitive to the attentional state of a conspecific and does this inform their foraging behaviour?

The results of a second experiment reported in Article III (*The Eyes and Ears are Visual Indicators of Attention in Domestic Horses*) indicate that horses are sensitive to the attentional state of a conspecific. This contributes to evidence (reviewed in the Introduction) that having the ability to recognise the attentional states of others is a

widespread skill, which is likely to have a long evolutionary history. This is perhaps unsurprising, given that this mechanism has the potential to have substantial survival benefits, for example advanced predator detection or indeed establishing whether a predator is attending to you (Burger et al., 1992, Burghardt and Greene, 1988, Emery, 2000, Freeberg et al., 2014, Hennig, 1977). Moreover, the ability to recognise the attentional state of another is a fundamental base that is necessary for more complex behaviours and cognitive processes, such as intentional communication (Aschersleben et al., 2008, Dennett, 1987, Schel et al., 2013). However, the evidence presented in Article III (*The Eyes and Ears are Visual Indicators of Attention in Domestic Horses*) also demonstrates that the attentional state of a conspecific influences horses' decisions about where to feed, and the scope of this skill has been more contentious. It has seemed strange that animals would have the ability to recognise the attentional state of another, but not use this skill to gain information about the environment. Particularly when this can be achieved through relatively simple mechanisms, such as stimulus enhancement, where the action of an individual draws the attention of the receiver to the stimulus (Goodwin, 2002). Potentially, previous conflicting results have been partly due to methodological flaws, which I have addressed by using a conspecific model in a naturalistic paradigm.

The ability to use the attentional state of another may also involve more complex systems, where information is communicated about the internal state of the signaller, their perspective, and/or the object of attention (Aschersleben et al., 2008, Bugnyar and Kotrschal, 2004, Okamoto-Barth et al., 2007). Previous studies examining the sensitivity of horses to human attention indicated that while adult horses are skilled at determining if a person is attending to them from subtle cues, they do not have this more advanced capacity (Krueger et al., 2011, Maros et al., 2008, McKinley and Sambrook, 2000, Proops and McComb, 2010, Proops et al., 2010). Horses seemed only to be able to use human communicative cues to inform them about their environment (for example the location of hidden food) if there was an element of stimulus enhancement. The experiment presented in Article III (*The Eyes and Ears are Visual Indicators of Attention in Domestic Horses*) does not establish whether horses were capable of this more complex social mechanism in their conspecific interactions, as the outcome could have easily been achieved through simple mechanisms – the horses

could have co-oriented with the model and then had their attention drawn to the corresponding food bucket.

Horses view humans as significant social objects, and in many of the tasks studying horse-human interactions the horses prioritised investigating the human model over the rest of the experimental set up. Furthermore, if the demonstrator was turned away from the horse, horses often positioned themselves in the humans' line of sight (Hausberger et al., 2008, Krueger et al., 2011, Maros et al., 2008, Proops et al., 2010). There is evidence that domestication is having a negative impact on problem solving abilities as animals are bred to rely on humans (Lesimple et al., 2012). Consequently the horses in these studies may have prioritised gaining the attention of the human experimenter over acquiring the food. Horses have evolved in an environment where they have a constant intake of abundant, low quality food and concentrated sources of high quality food are rare (although they are ubiquitous in the domestic environment). Therefore, it could be argued that the unusual behaviours demonstrated by horses in these tasks are highly adaptive and potentially indicative of more advanced cognition, as they were able to ignore a food source to prioritise the attentional state of a human. The lack of valuable, high-quality food in the natural environment also suggests that horses are unlikely to have evolved the ability to follow the attention of another specifically to enrich foraging, and that there are likely be other reasons. As prey animals, advanced predator detection is a highly relevant skill, but for horses the detection of social events may also be important; horses are known to interfere in third party interactions and there is some evidence to suggest that they perform third party consolation after conflict (Cozzi et al., 2010, Schneider and Krueger, 2012).

Article III (*The Eyes and Ears are Visual Indicators of Attention in Domestic Horses*) also demonstrates that horses require the detailed information in the facial expression of a conspecific, specifically involving the eyes and the ears, for the attentional state of a conspecific to influence their foraging behaviour. The ability to use signals from the eye area as an independent cue to attention is a hotly contested issue, with mixed results gained from a range of species (Emery, 2000, Kaminski et al., 2004, Proops and McComb, 2010, Proops et al., 2010, Rosati and Hare, 2009, Tempelmann et al., 2011). Overall, it seems that many animals are sensitive to the orientation of forward facing eyes and this appears to be an old ability that forms part of a rapid predator detection

system (Coss, 1978, Coss, 1979, Jones, 1980, Scaife, 1976). However, how animals with a differently shaped face gain information from the eyes of their conspecifics is a less considered question. It had been suggested that animals with laterally placed eyes would not be using cues from the eye area in their communication (Emery, 2000, Kaminski et al., 2005), but until now there had been no research that had tested this directly. It is not clear from Article III (*The Eyes and Ears are Visual Indicators of Attention in Domestic Horses*) exactly which cues from the eye area horses were attending to: potential signals may include the position of the eyeball in the socket, pupil size, and the presence of the inner brow raiser (AU101), but there may also be others of which we are unaware. The importance of the ears as well as the eyes for horses in following the attention of another highlights the need to consider a broad range of cues in the study of animal communication, and to keep in mind the fact that other species have different sensory systems to our own.

In many animals, if facial expressions coincide with the gaze cue, gaze following responses are enhanced (Bindemann et al., 2008, Lobmaier et al., 2008, Teufel et al., 2010). Many experimental paradigms testing the sensitivity of animals to the attentional state of another have provided cues in isolation and with a neutral facial expression. However, most signals will consist of multiple components that contribute some information to an overall message (Partan and Marler, 1999, Smith and Scott, 1997). The ability to take simultaneous cues into account, for example, both the attentional state of another and their emotional reaction to the object of their attention, would seem like a very efficient way of gaining relevant information. Whilst it is important to eliminate confounding variables and achieve clarity in experiments, we must also not ignore the complex interaction of behaviours and mental processes that will be representative of realistic cognition.

Here I have demonstrated that horses have one of the fundamental skills necessary to understand others and communicate with them effectively: the ability to recognise their attentional state and react accordingly. Next I build on this and discuss what the findings of this thesis tell us about the sensitivity of horses to more complex internal states of their conspecifics.

Are horses sensitive to the emotional state of a conspecific and does this inform their social behaviour?

Despite a wealth of literature discussing the production of expressions associated with emotional states in a range of species, surprisingly few experiments have investigated the ability to recognise emotion in non-human animals (Murphy et al., 2014). In humans, the production of facial expressions in response to emotional stimuli is rapid and difficult to suppress (Dimberg et al., 2000). Therefore facial expressions potentially provide a wealth of honest information about the internal state, motivations, and likely subsequent behaviour of another. Presumably in species for which life relies on interacting with other individuals who have rapidly changing needs and motivations, having an understanding of these facial expressions and what they might represent will be advantageous. Yet previous work that has investigated the perception of emotion from the facial expressions of others has overlooked how the information gained from these cues might be adaptive to the animal and give them advantages to functioning in their world (Waller and Micheletta, 2013).

The experiments reported in Article IV (*Functional use of Facial Expressions in a Non-Primate: Horses Discriminate Between Differing Emotions*) address this oversight by using naturalistic paradigms to investigate how the ability to recognise the emotional state of another might influence a significant social problem – do you approach an unfamiliar conspecific or not? This demonstrated that horses are able to discriminate cues to the emotional states of their conspecifics from their facial expressions and adjust their behaviour accordingly. These are skills that would presumably be seriously advantageous in real social interactions. Interestingly, these behavioural reactions were much stronger than the responses displayed to the emotional calls presented in Article II (*Auditory-Motor Representation for the Transfer of Information in the Horse*). It did seem that the vocalisations presented in Article II (*Auditory-Motor Representation for the Transfer of Information in the Horse*) were perceived differently and in line with their presumed affect (demonstrated by left eye/ear preferences for the negative call). However, the experimental paradigm employed for Article II (*Auditory-Motor Representation for the Transfer of Information in the Horse*) did not require the signal receivers to make an immediate social decision about approaching the novel horse, as they had to in Article IV (*Functional use of Facial Expressions in a Non-Primate: Horses Discriminate Between Differing Emotions*). If it had been possible to present

horses with the signal provider immediately after playing the affective calls, it would be interesting to note how the provision of previous information might have influenced the signal receiver's social behaviour.

There is a growing interest in what emotions non-human animals have and how these are expressed (Council of the European Union, 1997, Panksepp, 2005b, Panksepp, 2005a). In particular, there has been a recent drive to improve our knowledge of positive emotions in animals, which have long been neglected in comparison to work on negative emotional experiences (Boissy et al., 2007). However, facial expressions are often not considered in these efforts, and it has been reported that non-primates cannot display positive emotions in their face (Boissy et al., 2011, Tate et al., 2006). This is in spite of a distinct play face that is seen across a range of species and some other potential facial indicators of positive affect, such as eye narrowing and cheek raising (Ekman et al., 1990, Pellis and Pellis, 1997, Poole, 1978, Reed and Brown, 2000, Tabor, 2003). Articles II and IV (*Auditory-Motor Representations for the Transfer of Information in the Horse; Functional use of Facial Expressions in a Non-Primate: Horses Discriminate Between Differing Emotions*) report that horses did produce distinctive facial expressions in positive situations, including eye narrowing. Horses did also distinguish the positive facial expressions from the negative facial expressions in Article IV (*Functional use of Facial Expressions in a Non-Primate: Horses Discriminate Between Differing Emotions*), and horses reacted to the positive facial expressions in ways that indicate they were perceived in line with their presumed affect (e.g. more approach behaviour). Horses also distinguished the neutral face from the negative expression (although this discrimination was less strong than the positive-negative) but they did not show an obvious preference for the positive over the neutral. Furthermore, horses did not demonstrate multi-modal mirroring of the positive facial expression in Article II (*Auditory-Motor Representation for the Transfer of Information in the Horse*), as they did with the negative expression.

So, this work clearly demonstrates that horses can produce some facial expressions that are associated with positive emotional states, although future work is needed to investigate this area further. Potentially, in the paired choice task presented in Article IV (*Functional use of Facial Expressions in a Non-Primate: Horses Discriminate Between Differing Emotions – experiment 1*) there was no functional need for horses to

discriminate between the positive and the neutral facial expressions. However, chimpanzees also show no discrimination between a facial expression captured in positive context and a neutral face in computer based tasks, even though the chimpanzees are well trained on these matching tasks and could distinguish these faces from other expressions (Parr, 2003). This must lead us to question whether such expressions have differing representations in non-human animals. Even in humans, contentment or relaxation has been shown to elicit the same beneficial effects on the cardiovascular system as mild joy (Fredrickson, 2004, Fredrickson and Levenson, 1998). Potentially, these low arousal states have a roughly equivalent affective component, and are represented similarly in the brain.

Humans and some other primates have facial expressions that are similar in response to multiple stimuli, and it is thought that these expressions may convey non context-specific information about the motivational state of the signaller (Parr et al., 1998, Waal, 2006). Correspondingly, humans, chimpanzees, and rhesus macaques perceive emotional facial displays as belonging to distinct emotional categories, even though they are not given as discrete signals (Ekman et al., 2002, Hole and Bourne, 2010, Parr, 2011, Parr and Heintz, 2009). It has been suggested that the ability of animals to accurately decode the meaning of a facial expression across contexts requires a more challenging cognitive evaluation of the individual's motivation than simple learned associations of visual discrimination (Burgdorf and Panksepp, 2006, Liotti and Panksepp, 2004, Parr, 2003, Panksepp, 1998). In Article IV (*Functional use of Facial Expressions in a Non-Primate: Horses Discriminate Between Differing Emotions*) the facial expressions of the model horses were presented without any contextual cues, and thus it could be argued that the horses demonstrated a similar generalisation to extract non context-specific information about the motivational state of the signaller. It seems likely that these cognitive mechanisms have evolved under common selective pressures, such as need to interact with a network of associates in a complex and dynamic social environment. However, further work with a larger range of animals including both social and non-social species from diverse taxonomic families will be needed to fully understand the extent to which emotion recognition may have evolved in response to social pressures.

Section summary

Living in a community involves maintaining relationships with numerous other individuals in the face of competition for resources. Being able to ascertain the internal states, likely motivations, and future behaviours of others would presumably be advantageous for solving this social dilemma. Correspondingly, there is a growing body of evidence suggesting that social complexity and a need to maintain valuable relationships between individuals promotes the ability to gather, retain, and use detailed social knowledge. However, in order to gain insights into the complex social skills that some species display, we first need a full understanding of the basic processes through which animals gather information about their social counterparts. Using naturalistic paradigms that capture spontaneous reactions, I have demonstrated that horses are able to recognise key internal states from the detailed facial expressions of their conspecifics. Moreover, the information gained from these signals informs the behaviour of receivers in ways that offer them adaptive advantages.

In a social world, individuals need to continuously obtain information about the identities, emotions, and intentions of others from their actions. Here I have demonstrated that facial expressions contain meaningful information about the internal states of horses, which is accessible to their conspecifics. However ultimately, all behaviour can be studied in terms of its physiological underpinnings, and so this poses the interesting question of what are the physiological correlates of information transfer in these interactions? Studying this has the potential to give us insights into how animals experience their social world.

Mechanisms Underlying Social Cognition and Communication

Over recent decades the study of animal behaviour has undergone a significant transformation, with increasing discoveries and acceptance of abilities once considered uniquely human in a range of other species. These findings have generated considerable interest, and this has inspired researchers to try and uncover the extent and range of sophisticated behaviour and cognition. However, the fact that these complex cognitive constructs and behaviours are still not well understood is often overlooked. Moreover, some advanced faculties, such as empathy or altruism, are often categorised as distinct processes with very specific definitions, which may vary between researchers. It is

unlikely that this represents the true nature of behaviour and cognition in many cases. Evolution is a gradual process of modifications to existing capacities, derived from shared cellular structures across species. Therefore, there is a strong possibility that all complex cognition is rooted in basic processes that will be common to many animals. Moving towards understanding these fundamental mechanisms and how they may combine to develop the more complex processes that we see in some species may help us to gain a more comprehensive understanding of social cognition (LeDoux, 2012, Hecht et al., 2012, de Waal and Ferrari, 2010).

Moreover, gaining a better understanding of these basic components at the physiological level could offer us ways to measure and record behaviour more objectively, and develop more precise definitions of what constitutes complex behaviour (de Waal and Ferrari, 2010). Using this approach has taken scientists from having a vague concept of 'discrete inherited units' to understanding genetic information in exquisite detail (Cheney and Seyfarth, 2008, Slack, 2014). In my thesis I have adopted this bottom up approach, and in doing so have provided new insights into the fundamental processes underlying complex behaviour, cognition, and communication. Within the empirical chapters of the thesis I have investigated basic information transfer in spontaneous and naturalistic interactions, which has allowed us to assess the influence of this communication on the behaviour of horses in their environment. Through the development and application of an anatomically based tool for recording facial expressions, and the use of technology to record heart rate changes in response to stimuli, this work has also shed light on some of the physiological mechanisms that may be underpinning this information transfer and the behavioural responses that were displayed. These aspects will be discussed below, before drawing some final conclusions.

Perception-action representation for the transfer of information

There is growing support for a link between action perception and action execution. Studies indicate that observing an action can strengthen the motor representation responsible for performing it, and that performing an action can improve its visual discrimination (Casile and Giese, 2006, Stefan et al., 2005). This suggests that action perception and execution have shared neural representations and/or the representations

are activated simultaneously in either context. In line with this, there is a large body of evidence that has identified shared representations for both visual and auditory action perception and the sensory and motor representations of the experience of performing these actions (Aglioti and Pazzaglia, 2010, Banissy et al., 2010, Caramazza et al., 2014, Keysers and Gazzola, 2014, Kohler et al., 2002, Prather et al., 2008, Watkins et al., 2003). Auditory-motor representations are particularly interesting, because they indicate multi-modal integration of a more complex level than visual-motor mapping. In visual observation a correspondence could be established between low-level visual inputs and motor representations in the premotor cortex, as the content of the visual signal may include important information about the performance of that action, e.g. which effectors were used and what their position and speed were. In contrast, auditory signals provide much less information with which to establish a correspondence with a motor representation of an action (Aglioti and Pazzaglia, 2010). Critically, these representations are only activated by sounds that are related to an individual performing an action. Environmental sounds and phase scrambled versions of human action sounds are not associated with these mechanisms (Engel et al., 2009, Galati et al., 2008, Gazzola et al., 2006) Therefore, such processes seem to represent the neural merging of perception and action as a result of body mapping between individuals, and potentially offer a mechanism that underpins our understanding of the actions of others.

Neural representations

Some of these perception-action representations have been documented at the level of individual neurons. In humans and macaques neurons have been identified that fire selectively when the participants see an action being performed and perform the same action themselves (Keysers et al., 2003, Kohler et al., 2002, Mukamel et al., 2010). Auditory-motor ‘mirror neurons’ have also been identified in macaques and swamp sparrows, and in macaques some cells were identified that selectively responded to auditory inputs, visual inputs and the performance of the action (Keysers et al., 2003, Kohler et al., 2002, Prather et al., 2008). Whilst some of the cells involved in the perception-action processes appeared to be modality specific (i.e. responding to only visual or auditory perception), the multi-modal cells displayed an additive response to inputs from different modalities, suggesting they are involved in complex audio-visual-motor integration (Keysers et al., 2003). These differences within the general

perception-action mechanism are consistent with the idea that neurons with these shared activation properties are widespread throughout the brain and might contribute to many distinct neurological processes (Heyes, 2010). The possibility of matching the perception of a goal-directed act with its cortical motor representation could allow extraction of important information about another's action, prompting the suggestions that these neurons could be involved in action understanding in a range of situations.

Neurons with mirror properties have been identified that discriminate between similar actions that have different end goals e.g. either reaching for a piece of food and placing it in the mouth or reaching for the piece of food and placing it in a container close to the mouth. These cells displayed this selectivity close to the movement onset, before there were any obvious differences in the actions. Therefore, it is possible that these neurons help to infer another's intentions (Fogassi et al., 2005). Correspondingly, some of these neurons fire on the basis of partial information, coding the intention of a movement even if key parts of the action are occluded. For example, when pigtailed macaques viewed an experimenter interacting with objects with their hands (e.g. grasping, holding, or placing) neurons were recorded that showed mirror properties when the observers saw the entire action sequence and when the final stage of the action - during which the hand interacted with the object - was occluded. One interpretation of this is that on the basis of the observed part of the action sequence, the monkey understood the intention and the action being performed; thus, its understanding was reflected in the activity of the mirror neurons (Umilta et al., 2001).

From these results, it could be argued that the physiological basis of neural representations and information transfer are beginning to be understood. There is some debate about whether these neurons actively contribute to action understanding or whether their activity is only correlated with it, and a by-product from processes occurring in other parts of the brain that mediate action understanding (Heyes, 2010). However, there is also evidence that neural perception-action processes (although not mirror neurons specifically) do contribute to action understanding. In humans, activity in the sensorimotor cortices is evoked during the perception and production of affective vocalisations. Suppressing this sensorimotor activity reduces the ability to discriminate the emotional content of the call, but not other information, such as speaker identity

(Banissy et al., 2010). Therefore, it does seem that neural perception-action representations do have some role in understanding our social world.

Behavioural mirroring

These perception-action representations extend beyond the brain, and action observation leads to highly effector-specific and even muscle-specific modulations of corticospinal excitability. Transcranial magnetic stimulation (TMS) can be used to measure functional connections between performing a task and specific areas of the motor cortex. TMS can be applied to stimulate the motor cortex causing a contraction of specific, targeted muscles, which is measureable as a motor evoked potential (MEP). These MEPs serve as an index of motor excitability, and when performing a task enhances MEPs this provides evidence of a functional link between the task and the specific parts of motor cortex. For example, under TMS observation of an action can induce an increase in MEPs recorded from the same muscles that would be required to complete that action (Fadiga et al., 1995). Similar increases in motor evoked potentials were recorded from the tongue muscles of individuals listening to words that require tongue movements to articulate, and in the lip muscles of subjects listening to speech and non-verbal sounds and viewing speech related lip movements (Fadiga et al., 2002, Watkins et al., 2003). When perceiving an emotional facial expression, even when presented subliminally, people spontaneously produce the corresponding facial expression, and preventing this mimicry reduces the ability to recognise emotions in others (Dimberg et al., 2000, Lee et al., 2006). (Niedenthal, 2007). This suggests that this system is involved in emotion recognition. Indeed, behavioural mirroring is positively correlated with levels of empathy in humans, and dysfunction of the mirroring system is associated with social deficits (Dapretto et al., 2006, Iacoboni and Dapretto, 2006, Lakin et al., 2003).

Article II (*Auditory-Motor Representation for the Transfer of Emotional Information in the Horse*) now contributes to this field by demonstrating that rapid and multi-modal behavioural mirroring occurs in domestic horses in response to hearing affective vocalisations. This is the first demonstration of auditory-motor representation for the transfer of emotional information in a non-human animal. Simulation models of emotion recognition suggest that recognising and understanding emotions in others

requires individuals to map observed states onto their own representations of the corresponding emotional experiences (Adolphs, 2002, Goldman and Sripada, 2005, Keysers and Gazzola, 2014). Perception-action representations are a mechanism through which this correspondence can be established, and offer a way of representing social information (Gazzola et al., 2006). These processes could also offer a possible explanation for the inability of horses to recognise the attentional state of another horse when key features of the face were covered up, as reported in Article III (*The Eyes and Ears are Visual Indicators of Attention in Domestic Horses*). Potentially, obscuring key areas of the face prevented horses from enacting the necessary perception-action representations and therefore fully comprehending the stimuli.

It is unclear to what extent behavioural mirroring may be a stand-alone vicarious mechanism for understanding the actions of others, or is a by-product of neural activation in the motor cortices (Caramazza et al., 2014, Niedenthal, 2007). Some researchers consider that behavioural mirroring is the result of the neurological activation of mirror neurons, and that rapid mimicry, such as that produced by humans in response to emotional facial expressions, is indirect but compelling evidence that mirror neurons exist (Aglioti and Pazzaglia, 2010, Blakemore and Frith, 2005, Iacoboni and Dapretto, 2006). In this case the results reported in Article II (*Auditory-Motor Representation for the Transfer of Emotional Information in the Horse*) would be considered evidence for multi-modal mirror neurons in the horse.

The specific behaviours displayed in Article II (*Auditory-Motor Representation for the Transfer of Emotional Information in the Horse*) are especially interesting, as when humans adopt emotional postures and facial expressions they experience the associated emotion (Hess et al., 1992, Niedenthal, 2007). This emotional state matching (emotional contagion) is another perception-action mechanism that is thought to contribute to the transfer of emotional information and aid understanding of emotions and internal states in others (Lakin et al., 2003, Preston and De Waal, 2002, de Waal, 2008, de Waal and Ferrari, 2010). Although we do not have the evidence to conclude whether the production of affective facial expressions in Article II (*Auditory-Motor Representation for the Transfer of Emotional Information in the Horse*) generated emotional contagion, this mechanism was seen in horses when perceiving emotional facial expressions in Article IV (*Function Use of Facial Expressions in a Non-Primate: Horses Discriminate*

Between Differing Emotions). The increased heart rate (a key indicator of internal state) in response to the negative facial expressions demonstrated that viewing negative stimuli engendered a corresponding negative emotional state in the signal receiver. It must be noted that these physiological results alone do not necessarily indicate the activation of shared representations, and may reflect previously learned associations, for example, fear of a likely adverse encounter with a conspecific displaying a negative facial expression. However, both perception-action representation and fear from previously learned associations lead to the same end result: the creation of a negative emotional state from the perception of a negative facial expression.

In addition to a potential role in information transfer and the understanding of emotional states in others, this physiological state matching could underpin social behaviour and decision making through very simple processes. Emotional experiences are commonly agreed to have an adaptive value; negative emotions are typically characterised by the need to withdraw from a situation, whereas positive emotions engender a desire to approach (Adolphs, 2010, Cacioppo and Gardner, 1999, Davidson et al., 2000, Fredrickson, 2004, Frijda, 2009). Therefore, the propagation of a negative emotion in response to viewing the negative facial stimuli in Article IV (*Function Use of Facial Expressions in a Non-Primate: Horses Discriminate Between Differing Emotions*) may have generated the avoidance behaviours displayed by the stimuli receivers. This demonstrates how physiological processes can be driving seemingly complex social behaviours, and that by taking a bottom up approach we can begin to gain a more comprehensive understanding of behaviour.

Section summary

To understand communication and cognition we need to determine the fundamental mechanisms that underpin behaviour, and the correspondence between sensory and motor codes used to represent inputs. Perception-action representations are mechanisms through which this correspondence can be established, and offer a way of representing social information. Understanding this vicarious perception is providing novel insights in to the mechanisms of social cognition and offers us physiological pathways that might underpin behaviour and allow us to begin to understand more complex processes, such as empathy and perspective taking.

In this thesis I have demonstrated perception-action mechanisms for the transfer of emotional information in the domestic horse. These systems seem to contribute to a wide variety of processes in a range of species, from action perception in humans, to emotion recognition in horses, to song learning in birds. From this, it seems that these mechanisms have either co-evolved in social species, or can be traced back to the common ancestor of birds and mammals. Therefore, the possibility should be considered that all social understanding has a shared perception–action foundation, and that these are the building blocks from which complex cognition and communication has evolved in some species, such as humans. Humans are the result of a very long evolutionary history - by taking a broad view and studying a wide range of species, we can gain some insight into that history, and our shared heritage with the organisms that live alongside us.

Final Conclusions

In a wide range of taxa including primates, ungulates, carnivores, cetaceans, and insects, increased encephalisation over time is associated with increasing social group size, complexity, and stability (Connor et al., 1998, Dunbar, 2003, Dunbar, 1998, Dunbar and Shultz, 2007, Ehmer et al., 2001, Julian and Gronenberg, 2001, Pérez-Barbería et al., 2007, Shultz and Dunbar, 2010, Shultz and Dunbar, 2006). As brain tissue has high energy demands, it is thought that increases in brain capacity must also have evolutionary benefits. There is growing evidence that these advantages may be in the form of advanced socio-cognitive capacities, which assist social animals to respond to the challenges of living in complex social networks (Byrne and Whiten, 1988, Humphrey, 1976, Jolly, 1966). However, the corresponding comparative analysis of social cognition has not been conducted in such an extensive range of species. In this thesis I contribute to previous work by reporting new insights into social cognition and communication in a previously understudied species: the domestic horse.

Horses inhabit a relatively simple ecological environment, suggesting that any advanced cognition they display has not been in response to selection pressures from this domain. However, horses have rich social lives, and so it seems likely that the abilities that they possess probably developed to assist their survival within their complex social world. In the absence of domestic pressures, horses live in stable social groups within a larger,

dynamic community, which shares space and resources. Horses form strong social bonds with their conspecifics, and the ability to form these bonds is positively associated with fitness (Cameron et al., 2009, Nunez et al., 2014). It has been suggested that a range of skills would be necessary to function successfully and maintain relationships in such social networks, including: individual recognition; awareness of social standing (e.g. dominance) of yourself and others; and the ability to reconcile with valuable social counterparts after conflict (Byrne and Bates, 2007, Cheney and Seyfarth, 2008). Horses have been demonstrated to exhibit these skills, which are often considered characteristic of advanced social cognition (Cozzi et al., 2010, Emery, 2005, Emery, 2000, Krueger and Flauger, 2008, Waring, 2003). However, the key mechanisms through which horses (and other animals) gain and process social information are still poorly understood. Without this fundamental knowledge, we will not be able to gain a full understanding of complex social cognition and behaviour.

Facial expressions are rich sources of social information in humans and some other primates; however they are an understudied modality in most species. The research presented in this thesis has demonstrated that for horses, facial expressions are also an important source of social information that can influence their behaviour in adaptive ways. Moreover, there are a number of similarities between the facial repertoire of horses and some other animals, including humans and chimpanzees, despite the distant phylogenetic relationships. It seems that these common facial movements may appeal to sensory biases in the receiver, and thus promote efficient communication in social environments. This research supports the idea that advanced and sophisticated communicative repertories have been selected for in social species in response to the challenges that they face in navigating their social environment. Ultimately however, it will be difficult to fully evaluate these ideas without further research that enables us to make direct comparisons in a wider range of species with different levels of sociality. Moreover, future research should focus not only on social skills, but also investigate the extent to which advanced cognition is domain specific by conducting comparable social and non-social tasks. Perhaps Nicholas Humphrey (1976, p. 304) was not so far off the point when he suggested that there might be a need for, “a biologist to make it [her] task to explain why horses can’t learn mathematics.”

Crucially, in this thesis I have provided fundamental insights into the physiological

mechanisms underpinning social information transfer in horses. The investigation of these basic components of cognition could provide an understanding of how specific physiological and behavioural mechanisms contribute to the organisation of a given cognitive process and whether the same mechanisms operate across species. Then we could be in a position to consider how these might combine together or have been modified in some species to produce more unusual capacities. Thus it would be possible to tackle the study of complex cognitive processes, a full understanding of which is still eluding us, with an exploration of basic processes. This approach may offer a tangible way to quantify and test currently theoretical concepts, such as the existence of mental representations.

Finally, as a domestic animal, understanding more about horses, their communication, and their emotional lives will have significant welfare implications. We now know that horses express their internal states through facial expressions, and we have a tool through which these facial expressions can be accurately recorded and quantified in future work. Researchers are beginning to study the expression of pain in the faces of horses (Dalla Costa et al., 2014, Gleerup et al., 2015) however the identification of cues that are associated with a wide range of situations, and particularly positive situations, will also be important. This must be done alongside the development of novel experimental paradigms that allow us to gain insights into how the horses perceive these emotional states. Furthermore, as it is clear that facial expressions are key for communicating with other horses, future work should identify whether the tendency to cover key parts of the faces of horses affects their social behaviour and subsequent welfare in the domestic environment.

References

Adolphs, R. 2002. Neural systems for recognizing emotion. *Current opinion in neurobiology,* 12**,** 169-177.

Adolphs, R. 2010. Emotion. *Current Biology,* 20**,** R549-R552.

Aglioti, S. M. & Pazzaglia, M. 2010. Representing actions through their sound. *Experimental brain research,* 206**,** 141-51.

Andrew, R. J. 1963. Evolution of Facial Expression. *Science,* 142**,** 1034-1041.

Aschersleben, G., Hofer, T. & Jovanovic, B. 2008. The link between infant attention to goal-directed action and later theory of mind abilities. *Developmental science,* 11**,** 862-8.

Aureli, F., Schaffner, C m., Boesch, C., Bearder, S k., Call, J., Chapman, C a., Connor, R., Fiore, A d., Dunbar, R i. M., Henzi, S. P., Holekamp, K., Korstjens, A h., Layton, R., Lee, P., Lehmann, J., Manson, J h., Ramos- Fernandez, G., Strier, K b. & Van Schaik, C p. 2008. Fission- Fusion Dynamics: New Research Frameworks. *Current Anthropology,* 49**,** 627-654.

Banissy, M. J., Sauter, D. A., Ward, J., Warren, J. E., Walsh, V. & Scott, S. K. 2010. Suppressing sensorimotor activity modulates the discrimination of auditory emotions but not speaker identity. *The Journal of Neuroscience,* 30**,** 13552-7.

Bearder, S. K., Nekaris, K. a. I. & Curtis, D. J. 2006. A Re-Evaluation of the Role of Vision in the Activity and Communication of Nocturnal Primates. *Folia Primatologica,* 77**,** 50-71.

Bindemann, M., Mike Burton, A. & Langton, S. R. H. 2008. How do eye gaze and facial expression interact? *Visual Cognition,* 16**,** 708-733.

Blakemore, S. J. & Frith, C. 2005. The role of motor contagion in the prediction of action. *Neuropsychologia,* 43**,** 260-7.

Boissy, A., Aubert, A., Desire, L., Greiveldinger, L., Delval, E. & Veissier, I. 2011. Cognitive sciences to relate ear postures to emotions in sheep. *Animal Welfare,* 20**,** 47.

Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I. & Aubert, A. 2007. Assessment of positive emotions in animals to improve their welfare. *Physiology & Behavior,* 92**,** 375-97.

Bolwig, N. 1964. Facial Expression in Primates With Remarks On a Parallel Development in Certain Carnivores (a Preliminary Report On Work in Progress). *Behaviour,* 22**,** 167-192.

Bruce, V. & Young, A. 2013. *Face Perception,* Taylor & Francis.

Bugnyar, T. & Kotrschal, K. 2004. Leading a conspecific away from food in ravens (Corvus corax)? *Animal cognition,* 7**,** 69-76.

Burgdorf, J. & Panksepp, J. 2006. The neurobiology of positive emotions. *Neuroscience and biobehavioral reviews,* 30**,** 173-87.

Burger, J., Gochfeld, M. & Murray, B. G. 1992. Risk discrimination of eye contact and directness of approach in black iguanas (Ctenosaura similis). *Journal of Comparative Psychology,* 106**,** 97.

Burghardt, G. M. & Greene, H. W. 1988. Predator simulation and duration of death feigning in neonate hognose snakes. *Animal Behaviour,* 36**,** 1842-1844.

Burrows, A. M. 2008. The facial expression musculature in primates and its evolutionary significance. *BioEssays,* 30**,** 212-225.

Burrows, A. M., Diogo, R., Waller, B. M., Bonar, C. J. & Liebal, K. 2011. Evolution of the Muscles of Facial Expression in a Monogamous Ape: Evaluating the

Relative Influences of Ecological and Phylogenetic Factors in Hylobatids. *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology,* 294**,** 645-663.

Burrows, A. M. & Smith, T. D. 2003. Muscles of facial expression in Otolemur, with a comparison to lemuroidea. *The anatomical record. Part A, Discoveries in molecular, cellular, and evolutionary biology,* 274**,** 827-36.

Burrows, A. M., Waller, B. M. & Parr, L. A. 2009. Facial musculature in the rhesus macaque (Macaca mulatta): evolutionary and functional contexts with comparisons to chimpanzees and humans. *Journal of Anatomy,* 215**,** 320-334.

Burrows, A. M., Waller, B. M., Parr, L. A. & Bonar, C. J. 2006. Muscles of facial expression in the chimpanzee (Pan troglodytes): descriptive, comparative and phylogenetic contexts. *Journal of Anatomy,* 208**,** 153-167.

Byrne, R. W. 1995. *The Thinking Ape: Evolutionary Origins of Intelligence*, Oxford University Press.

Byrne, R. W. & Bates, L. A. 2007. Sociality, evolution and cognition. *Current biology : CB,* 17**,** R714-23.

Byrne, R. W. & Whiten, A. 1988. *Machiavellian Intelligence: Social Expertise and the Evolution of Intellect in Monkeys, Apes, and Humans*, Clarendon Press.

Cacioppo, J. T. & Gardner, W. L. 1999. Emotion. *Annual Review of Psychology,* 50**,** 191-214.

Caeiro, C. C., Waller, B. M., Zimmermann, E., Burrows, A. M. & Davila-Ross, M. 2012. OrangFACS: A Muscle-Based Facial Movement Coding System for Orangutans (Pongo spp.). *International Journal of Primatology,* 34**,** 115-129.

Cameron, E. Z., Setsaas, T. H. & Linklater, W. L. 2009. Social bonds between unrelated females increase reproductive success in feral horses. *Proceedings of the National Academy of Sciences of the United States of America,* 106**,** 13850-3.

Caramazza, A., Anzellotti, S., Strnad, L. & Lingnau, A. 2014. Embodied cognition and mirror neurons: A critical assessment. *Annual Review of Neuroscience,* 37**,** 1-15.

Casile, A. & Giese, M. A. 2006. Nonvisual motor training influences biological motion perception. *Current Biology,* 16**,** 69-74.

Cheney, D. L. & Seyfarth, R. M. 2008. *Baboon Metaphysics: The Evolution of a Social Mind*, University of Chicago Press.

Chevalier-Skolnikoff, S. 1973. Facial expressions of emotion in non-human primates. *In:* Ekman, P. (ed.) *Darwin and Facial Expressions.* New York: Acadamy Press.

Connor, R. C., Mann, J., Tyack, P. L. & Whitehead, H. 1998. Social evolution in toothed whales. *Trends in ecology & evolution,* 13**,** 228-232.

Coss, R. G. 1978. Perceptual determinants of gaze aversion by the lesser mouse lemur (Microcebus murinus), the role of two facing eyes. *Behaviour,* 64**,** 248-269.

Coss, R. G. 1979. Delayed plasticity of an instinct: recognition and avoidance of 2 facing eyes by the jewel fish. *Developmental psychobiology,* 12**,** 335-345.

Council of the European Union. 1997. Treaty on European Union (Consolidated Version), Treaty of Amsterdam.

Cozzi, A., Sighieri, C., Gazzano, A., Nicol, C. J. & Baragli, P. 2010. Post-conflict friendly reunion in a permanent group of horses (Equus caballus). *Behavioural processes,* 85**,** 185-90.

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E. & Leach, M. C. 2014. Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration. *PLoS one,* 9**,** e92281.

Dapretto, M., Davies, M. S., Pfeifer, J. H., Scott, A. A., Sigman, M., Bookheimer, S. Y. & Iacoboni, M. 2006. Understanding emotions in others: mirror neuron dysfunction in children with autism spectrum disorders. *Nature neuroscience,* 9**,** 28-30.

Darwin, C. 1999. *The Expression of the Emotions in Man and Animals,* London, UK, Fontana Press.

Davidson, R. J., Jackson, D. C. & Kalin, N. H. 2000. Emotion, plasticity, context, and regulation: perspectives from affective neuroscience. *Psychological Bulletin,* 126**,** 890.

Dawkins, M. S. & Guildford, T. 1991. The corruption of honest signalling. *Animal Behaviour,* 41, 865-873.

De Waal, F. B. 2008. Putting the altruism back into altruism: the evolution of empathy. *Annual Review of Psychology,* 59**,** 279-300.

De Waal, F. B. & Ferrari, P. F. 2010. Towards a bottom-up perspective on animal and human cognition. *Trends in cognitive sciences,* 14**,** 201-7.

Dennett, D. 1987. *The intentional stance,* Cambridge, MA, USA., MIT Press.

Dimberg, U., Thunberg, M. & Elmehed, K. 2000. Unconscious Facial Reactions to Emotional Facial Expressions. *Psychological science,* 11**,** 86-89.

Dobson, S. D. 2009a. Allometry of facial mobility in anthropoid primates: implications for the evolution of facial expression. *American journal of physical anthropology,* 138**,** 70-81.

Dobson, S. D. 2009b. Socioecological correlates of facial mobility in nonhuman anthropoids. *American journal of physical anthropology,* 139**,** 413-20.

Dobson, S. D. 2012. Face to face with the social brain. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 367**,** 1901-8.

Dunbar, R. I. 1998. The social brain hypothesis. *Brain,* 9**,** 178-190.

Dunbar, R. I. 2003. The social brain: mind, language, and society in evolutionary perspective. *Annual Review of Anthropology***,** 163-181.

Dunbar, R. I. & Shultz, S. 2007. Evolution in the social brain. *Science,* 317**,** 1344-7.

Dunn, P. O., Cockburn, A. & Mulder, R. A. 1995. *Fairy-Wren Helpers often Care for Young to Which they are Unrelated*.

Ehmer, B., Reeve, H. K. & Hoy, R. R. 2001. Comparison of brain volumes between single and multiple foundresses in the paper wasp Polistes dominulus. *Brain, behavior and evolution,* 57**,** 161-168.

Ekman, P., Davidson, R. J. & Friesen, W. V. 1990. The Duchenne smile: emotional expression and brain physiology. II. *J Pers Soc Psychol,* 58**,** 342-53.

Ekman, P. & Friesen, W. V. 1971. Constants across cultures in the face and emotion. *Journal of personality and social psychology,* 17**,** 124.

Ekman, P., Friesen, W. V. & Hager, J. C. 2002. *Facial Action Coding System,* Salt Lake City, Research Nexus.

Ekman, P. & Oster, H. 1979. Facial Expressions of Emotion. *Annual Review of Psychology,* 30**,** 527-554.

Ekman, P., Sorenson, E. R. & Friesen, W. V. 1969. Pan-Cultural Elements in Facial Displays of Emotion. *Science,* 164**,** 86-88.

Emery, N. J. 2000. The eyes have it: the neuroethology, function and evolution of social gaze. *Neuroscience and Biobehavioral Reviews,* 24**,** 581-604.

Emery, N. J. 2005. The evolution of social cognition. *In:* Easton, A. & Emery, N. J. (eds.) *The Cognitive Neuroscience of Social Behaviour*. Hove, UK: Psychology Press.

Engel, L. R., Frum, C., Puce, A., Walker, N. A. & Lewis, J. W. 2009. Different categories of living and non-living sound-sources activate distinct cortical networks. *NeuroImage,* 47**,** 1778-1791.

Fadiga, L., Craighero, L., Buccino, G. & Rizzolatti, G. 2002. Speech listening specifically modulates the excitability of tongue muscles: a TMS study. *European Journal of Neuroscience,* 15**,** 399-402.

Fadiga, L., Fogassi, L., Pavesi, G. & Rizzolatti, G. 1995. Motor facilitation during action observation: a magnetic stimulation study. *Journal of neurophysiology,* 73**,** 2608-2611.

Flack, J. C. & De Waal, F. 2007. Context modulates signal meaning in primate communication. *Proceedings of the National Academy of Sciences of the United States of America,* 104**,** 1581-6.

Fogassi, L., Ferrari, P. F., Gesierich, B., Rozzi, S., Chersi, F. & Rizzolatti, G. 2005. Parietal Lobe: From Action Organization to Intention Understanding. *Science,* 308**,** 662-667.

Fox, M. W. 1970. A Comparative Study of the Development of Facial Expressions in Canids; Wolf, Coyote and Foxes. *Behaviour,* 36**,** 49-73.

Fredrickson, B. L. 2004. The broaden-and-build theory of positive emotions. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 359**,** 1367-78.

Fredrickson, B. L. & Levenson, R. W. 1998. Positive Emotions Speed Recovery from the Cardiovascular Sequelae of Negative Emotions. *Cognition & emotion,* 12**,** 191-220.

Freeberg, T. M., Krama, T., Vrublevska, J., Krams, I. & Kullberg, C. 2014. Tufted titmouse (Baeolophus bicolor) calling and risk-sensitive foraging in the face of threat. *Animal cognition,* 17**,** 1341-1352.

Frijda, N. H. 2009. Emotion Experience and its Varieties. *Emotion Review,* 1**,** 264-271.

Galati, G., Committeri, G., Spitoni, G., Aprile, T., Di Russo, F., Pitzalis, S. & Pizzamiglio, L. 2008. A selective representation of the meaning of actions in the auditory mirror system. *NeuroImage,* 40**,** 1274-1286.

Gazzola, V., Aziz-Zadeh, L. & Keysers, C. 2006. Empathy and the somatotopic auditory mirror system in humans. *Current biology : CB,* 16**,** 1824-9.

Gleerup, K. B., Forkman, B., Lindegaard, C. & Andersen, P. H. 2015. An equine pain face. *Veterinary anaesthesia and analgesia,* 42**,** 103-14.

Goldman, A. I. & Sripada, C. S. 2005. Simulationist models of face-based emotion recognition. *Cognition,* 94**,** 193-213.

Goody, E. N. 1997. Social intelligence and language: Another Rubicon? *In:* Whiten, A. & Byrne, R. W. (eds.) *Machiavellian Inteligence II. Extensions and Evaluations.* Cambridge, UK: Cambridge University Press.

Harnad, S. 2003. Categorical perception. *Encyclopedia of cognitive science,* 67.

Hausberger, M., Roche, H., Henry, S. & Visser, E. K. 2008. A review of the human–horse relationship. *Applied Animal Behaviour Science,* 109**,** 1-24.

Hecht, E. E., Patterson, R. & Barbey, A. K. 2012. What can other animals tell us about human social cognition? An evolutionary perspective on reflective and reflexive processing. *Frontiers in human neuroscience,* 6**,** 224.

Hennig, C. W. 1977. Effects of simulated predation on tonic immobility in Anolis carolinensis: The role of eye contact. *Bulletin of the Psychonomic Society,* 9**,** 239-242.

Hess, U., Kappas, A., Mchugo, G. J., Lanzetta, J. T. & Kleck, R. E. 1992. The facilitative effect of facial expression on the self-generation of emotion. *International Journal of Psychophysiology,* 12**,** 251-265.

Heyes, C. 2010. Where do mirror neurons come from? *Neuroscience and biobehavioral reviews,* 34**,** 575-83.

Hole, G. & Bourne, V. 2010. *Face Processing: Psychological, Neuropsychological, and Applied Perspectives,* OUP Oxford.

Huber, E. 1931. *Evolution of facial musculature and expression,* Baltimore, John Hopkins Press.

Humphrey, N. 1976. The social function of intellect. *In:* Bateson, P. P. G. & Hinde, R. A. (eds.) *Growing Points in Ethology.* Cambridge: Cambridge University Press.

Iacoboni, M. & Dapretto, M. 2006. The mirror neuron system and the consequences of its dysfunction. *Nature reviews. Neuroscience,* 7**,** 942-51.

Jolly, A. 1966. Lemur social behaviour and primate intelligence. *Science,* 153**,** 510-506.

Jones, R. 1980. Reactions of male domestic chicks to two-dimensional eye-like shapes. *Animal Behaviour,* 28**,** 212-218.

Julian, G. E. & Gronenberg, W. 2001. Reduction of brain volume correlates with behavioral changes in queen ants. *Brain, behavior and evolution,* 60**,** 152-164.

Kaminski, J., Call, J. & Tomasello, M. 2004. Body orientation and face orientation: two factors controlling apes' behavior from humans. *Animal cognition,* 7**,** 216-23.

Kaminski, J., Riedel, J., Call, J. & Tomasello, M. 2005. Domestic goats, Capra hircus, follow gaze direction and use social cues in an object choice task. *Animal Behaviour,* 69**,** 11-18.

Keysers, C. & Gazzola, V. 2014. *Hebbian learning and predictive mirror neurons for actions, sensations and emotions*.

Keysers, C., Kohler, E., Umiltá, M. A., Nanetti, L., Fogassi, L. & Gallese, V. 2003. Audiovisual mirror neurons and action recognition. *Experimental brain research,* 153**,** 628-636.

Kobayashi, H. & Kohshima, S. 1997. Unique morphology of the human eye. *Nature,* 387**,** 767-768.

Kobayashi, H. & Kohshima, S. 2001. Unique morphology of the human eye and its adaptive meaning: comparative studies on external morphology of the primate eye. *Journal of human evolution,* 40**,** 419-435.

Kohler, E., Keysers, C., Umilta, M. A., Fogassi, L., Gallese, V. & Rizzolatti, G. 2002. Hearing sounds, understanding actions: action representation in mirror neurons. *Science,* 297**,** 846-8.

Krueger, K. & Flauger, B. 2008. Social feeding decisions in horses (Equus caballus). *Behavioural processes,* 78**,** 76-83.

Krueger, K., Flauger, B., Farmer, K. & Maros, K. 2011. Horses (Equus caballus) use human local enhancement cues and adjust to human attention. *Animal cognition,* 14**,** 187-201.

Lakin, J., Jefferis, V., Cheng, C. & Chartrand, T. 2003. The Chameleon Effect as Social Glue: Evidence for the Evolutionary Significance of Nonconscious Mimicry. *Journal of nonverbal behavior,* 27**,** 145-162.

Ledoux, J. 2012. Rethinking the emotional brain. *Neuron,* 73**,** 653-76.

Lee, T. W., Josephs, O., Dolan, R. J. & Critchley, H. D. 2006. Imitating expressions: emotion-specific neural substrates in facial mimicry. *Social Cognitive and Affective Neuroscience,* 1**,** 122-35.

Leiner, L. & Fendt, M. 2011. Behavioural fear and heart rate responses of horses after exposure to novel objects: Effects of habituation. *Applied Animal Behaviour Science,* 131**,** 104-109.

Lesimple, C., Sankey, C., Richard, M. A. & Hausberger, M. 2012. Do horses expect humans to solve their problems? *Frontiers in psychology,* 3**,** 306.

Liem, K. F. & Walker, W. F. 2001. *Functional anatomy of the vertebrates: an evolutionary perspective*, Harcourt College Publishers.

Liotti, M. & Panksepp, J. 2004. Imaging human emotions and affective feelings, Implications for biological pscyhiatry. *In:* Panksepp, J. (ed.) *Textbook of Biological Psychiatry.* Hoboken, NJ: Wiley.

Lobmaier, J. S., Tiddeman, B. P. & Perrett, D. I. 2008. Emotional expression modulates perceived gaze direction. *Emotion,* 8**,** 573-7.

Maros, K., Gacsi, M. & Miklosi, A. 2008. Comprehension of human pointing gestures in horses (Equus caballus). *Animal cognition,* 11**,** 457-66.

Mccomb, K. & Semple, S. 2005. *Coevolution of vocal communication and sociality in primates*.

Mcdonnell, S. 2003. *The Equid Ethogram: A Practical Field Guide to Horse Behavior*, ECLIPSE Press.

Mckinley, J. & Sambrook, T. D. 2000. Use of human-given cues by domestic dogs (Canis familiaris) and horses (Equus caballus). *Animal cognition,* 3**,** 13-22.

Mukamel, R., Ekstrom, A. D., Kaplan, J., Iacoboni, M. & Fried, I. 2010. Single-neuron responses in humans during execution and observation of actions. *Current biology : CB,* 20**,** 750-6.

Murphy, E., Nordquist, R. E. & Van Der Staay, F. J. 2014. A review of behavioural methods to study emotion and mood in pigs, Sus scrofa. *Applied Animal Behaviour Science,* 159**,** 9-28.

Niedenthal, P. M. 2007. Embodying Emotion. *Science,* 316**,** 1002-1005.

Nunez, C. M. V., Adelman, J. S. & Rubenstein, D. I. 2014. Sociality increases juvenile survival after a catastrophic event in the feral horse (Equus caballus). *Behavioral Ecology,* 26**,** 138-147.

Okamoto-Barth, S., Call, J. & Tomasello, M. 2007. Great apes' understanding of other individuals' line of sight. *Psychological science,* 18**,** 462-468.

Panksepp, J. 1998. *Affective Neuroscience: The Foundations of Human and Animal Emotions*, Oxford University Press.

Panksepp, J. 2005a. Affective consciousness: Core emotional feelings in animals and humans. *Consciousness and Cognition,* 14**,** 30-80.

Panksepp, J. 2005b. Psychology. Beyond a joke: from animal laughter to human joy? *Science,* 308**,** 62-3.

Parr, L. A. 2003. The Discrimination of Faces and Their Emotional Content by Chimpanzees (Pan troglodytes). *Annals of the New York Academy of Sciences,* 1000**,** 56-78.

Parr, L. A. 2011. The evolution of face processing in primates. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 366**,** 1764-77.

Parr, L. A., Cohen, M. & Waal, F. 2005. Influence of Social Context on the Use of Blended and Graded Facial Displays in Chimpanzees. *International Journal of Primatology,* 26**,** 73-103.

Parr, L. A. & Heintz, M. 2009. Facial expression recognition in rhesus monkeys, Macaca mulatta. *Animal Behaviour*, 77**,** 1507-1513.

Parr, L. A., Hopkins, W. D. & De Waal, F. 1998. The perception of facial expressions by chimpanzees, Pan troglodytes. *Evolution of Communication,* 2**,** 1-23.

Parr, L. A., Waller, B. M., Burrows, A. M., Gothard, K. M. & Vick, S. J. 2010. MaqFACS: A muscle-based facial movement coding system for the rhesus macaque. *American journal of physical anthropology,* 143**,** 625-30.

Parr, L. A., Waller, B. M., Vick, S. J. & Bard, K. A. 2007. Classifying chimpanzee facial expressions using muscle action. *Emotion,* 7**,** 172-81.

Partan, S. & Marler, P. 1999. Communication Goes Multimodal. *Science,* 283**,** 1272-1273.

Pellis, S. M. & Pellis, V. C. 1997. Targets, tactics, and the open mouth face during play fighting in three species of primates. *Aggressive Behavior,* 23**,** 41-57.

Pérez-Barbería, F. J., Shultz, S. & Dunbar, R. I. M. 2007. Evidence for coevolution of sociality and relative brain size in three orders of mammals. *Evolution,* 61**,** 2811-2821.

Poole, T. B. 1978. Analysis of social play in polecats (*Mustelidae*) with comments on form and evolutionary history of open mouth play face. *Animal Behaviour,* 26**,** 36-49.

Prather, J. F., Peters, S., Nowicki, S. & Mooney, R. 2008. Precise auditory-vocal mirroring in neurons for learned vocal communication. *Nature,* 451**,** 305-10.

Premack, D. & Woodruff, G. 1978. Does the chimpanzee have a theory of mind? *Behavioral and Brain Sciences,* 1**,** 515-526.

Preston, S. D. & De Waal, F. 2002. Empathy: Its ultimate and proximate bases. *Behavioral and Brain Sciences,* 25**,** 1-20.

Preuschoft, S. & Van Hooff, J. A. 1997. The social function of" smile" and" laughter": Variations across primate species and societies.

Proops, L. & Mccomb, K. 2010. Attributing attention: the use of human-given cues by domestic horses (Equus caballus). *Animal cognition,* 13**,** 197-205.

Proops, L., Walton, M. & Mccomb, K. 2010. The use of human-given cues by domestic horses, Equus caballus, during an object choice task. *Animal Behaviour,* 79**,** 1205-1209.

Pusey, A. & Packer, C. 2003. The Ecology of Relationships. *In:* Krebs, J. & Davies, N. (eds.) *Behavioural Ecology.* Oxford: Blackwell Scientific Publication.

Reby, D. & Mccomb, K. 2003. Vocal communication and reproduction in deer. *Advances in the Study of Behavior,* 33**,** 231-264.

Reed, T. & Brown, M. 2000. The expression of care in the rough and tumble play of boys. *Journal of Research in Childhood Education,* 15**,** 104-116.

Rinn, W. E. 1984. The neuropsychology of facial expression: A review of the neurological and psychological mechanisms for producing facial expressions. *Psychological Bulletin,* 95**,** 52-77.

Rosati, A. G. & Hare, B. 2009. Looking past the model species: diversity in gaze-following skills across primates. *Current opinion in neurobiology,* 19**,** 45-51.

Scaife, M. 1976. The response to eye-like shapes by birds II. The importance of staring, pairedness and shape. *Animal Behaviour,* 24**,** 200-206.

Schel, A. M., Townsend, S. W., Machanda, Z., Zuberbühler, K. & Slocombe, K. E. 2013. Chimpanzee alarm call production meets key criteria for intentionality. *PLoS one,* 8**,** e76674.

Schmidt, K. L. & Cohn, J. F. 2001. Human facial expressions as adaptations: Evolutionary questions in facial expression research. *American journal of physical anthropology,* Suppl 33**,** 3-24.

Schneider, G. & Krueger, K. 2012. Third-party interventions keep social partners from exchanging affiliative interactions with others. *Animal Behaviour,* 83**,** 377-387.

Seyfarth, R. M. & Cheney, D. L. 2003. Signalers and receivers in animal communication. *Annual Review of Psychology,* 54**,** 145-73.

Seyfarth, R. M., Cheney, D. L., Bergman, T. J., Fischer, J., Zuberbühler, K. & Hammerschmidt, K. 2010. The central importance of information in studies of animal communication. *Animal Behaviour,* 80**,** 3-8.

Shultz, S. & Dunbar, R. 2010. Encephalization is not a universal macroevolutionary phenomenon in mammals but is associated with sociality. *Proceedings of the National Academy of Sciences,* 107**,** 21582-21586.

Shultz, S. & Dunbar, R. I. 2006. Both social and ecological factors predict ungulate brain size. *Proceedings. Biological sciences / The Royal Society,* 273**,** 207-15.

Silk, J. B., Beehner, J. C., Bergman, T. J., Crockford, C., Engh, A. L., Moscovice, L. R., Wittig, R. M., Seyfarth, R. M. & Cheney, D. L. 2009. The benefits of social capital: close social bonds among female baboons enhance offspring survival. *Proceedings. Biological sciences / The Royal Society,* 276**,** 3099-104.

Silk, J. B., Beehner, J. C., Bergman, T. J., Crockford, C., Engh, A. L., Moscovice, L. R., Wittig, R. M., Seyfarth, R. M. & Cheney, D. L. 2010. Strong and consistent social bonds enhance the longevity of female baboons. *Current biology : CB,* 20**,** 1359-61.

Slack, J. 2014. *Genes: A Very Short Introduction*, Oxford University Press.

Smith, C. A. & Scott, H. S. 1997. A componential approach to the meaning of facial expressions. *In:* Russell, J. A. & Fernåndez-Dols, J. M. (eds.) *The Psychology of Facial Expression.* Cambridge: Cambridge University Press.

Stefan, K., Cohen, L. G., Duque, J., Mazzocchio, R., Celnik, P., Sawaki, L., Ungerleider, L. & Classen, J. 2005. Formation of a motor memory by action observation. *The Journal of Neuroscience*, 25**,** 9339-9346.

Tabor, R. 2003. *Understanding Cat Behavior*, F+W Media.

Tate, A. J., Fischer, H., Leigh, A. E. & Kendrick, K. M. 2006. Behavioural and neurophysiological evidence for face identity and face emotion processing in animals. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 361**,** 2155-72.

Taylor, A. M. & Reby, D. 2010. The contribution of source-filter theory to mammal vocal communication research. *Journal of Zoology,* 280**,** 221-236.

Tempelmann, S., Kaminski, J. & Liebal, K. 2011. Focus on the essential: all great apes know when others are being attentive. *Animal cognition,* 14**,** 433-439.

Teufel, C., Gutmann, A., Pirow, R. & Fischer, J. 2010. Facial expressions modulate the ontogenetic trajectory of gaze-following among monkeys. *Developmental science,* 13**,** 913-922.

Umilta, M. A., Kohler, E., Gallese, V., Fogassi, L., Fadiga, L., Keysers, C. & Rizzolatti, G. 2001. I know what you are doing: A neurophysiological study. *Neuron,* 31**,** 155-165.

Vick, S. J., Waller, B. M., Parr, L. A., Smith Pasqualini, M. C. & Bard, K. A. 2007. A Cross-species Comparison of Facial Morphology and Movement in Humans and Chimpanzees Using the Facial Action Coding System (FACS). *Journal of nonverbal behavior,* 31**,** 1-20.

Waal, F. B. M. 2006. Darwin's Legacy and the Study of Primate Visual Communication. *Annals of the New York Academy of Sciences,* 1000**,** 7-31.

Waller, B. M., Cray, J. J. & Burrows, A. M. 2008. Selection for universal facial emotion. *Emotion,* 8**,** 435-9.

Waller, B. M. & Dunbar, R. I. 2005. Differential behavioural effects of silent bared teeth display and relaxed open mouth display in chimpanzees. *Ethology,* 111**,** 129-142.

Waller, B. M., Lembeck, M., Kuchenbuch, P., Burrows, A. M. & Liebal, K. 2012. GibbonFACS: A Muscle-Based Facial Movement Coding System for Hylobatids. *International Journal of Primatology,* 33**,** 809-821.

Waller, B. M. & Micheletta, J. 2013. Facial Expression in Nonhuman Animals. *Emotion Review,* 5**,** 54-59.

Waller, B. M., Peirce, K., Caeiro, C. T. C., Scheider, L., Burrows, A. M., Mccune, S. & Kaminski, J. 2013. Paedomorphic Facial Expressions Give Dogs a Selective Advantage. *PLoS one,* 8**,** e82686.

Waller, B. M., Vick, S.J., Parr, L.A., Bard, K.A., Smith Pasqualini, M.C., Gothard, K. & Fuglevand, A. 2006. Intramusclar stimulation of facial muscles in humans and chimpanzees: Duchenne revisted. . *Emotion,* 6**,** 387-382.

Waring, G. H. 2003. *Horse Behavior,* Noyes Publications/William Andrew Pub.

Watkins, K. E., Strafella, A. P. & Paus, T. 2003. Seeing and hearing speech excites the motor system involved in speech production. *Neuropsychologia,* 41**,** 989-994.

Whalen, P. J., Kagan, J., Cook, R. G., Davis, F. C., Kim, H., Polis, S., Mclaren, D. G., Somerville, L. H., Mclean, A. A., Maxwell, J. S. & Johnstone, T. 2004. Human Amygdala Responsivity to Masked Fearful Eye Whites. *Science,* 306**,** 2061.